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Effects of Atmospheric Pollutants on Human Physiologic Function

Professional Staff Assn. of the Rancho Los Amigos Hospital, Inc.

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EFFECT OF ATMOSPHERIC POLLUTANTS ON HUMAN PHYSIOLOGIC FUNCTION

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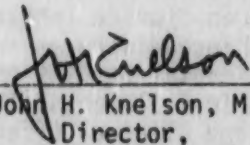
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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory develops and revises air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is preparing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

The studies reported here were initiated under EPA support in 1971. In subsequent years, support for the overall effort was also provided by other organizations -- the National Heart and Lung Institute and the California Air Resources Board. Since the investigations funded by all three agencies are closely interrelated and relevant to each other, information from all is included here. No attempt is made to distinguish sources of support; instead, the presentation attempts to give maximum scientific coherence to the overall findings. The bulk of the report (Section B) consists of publication reprints and preprints on specific effects of air pollution on human health; these are preceded by a summary and overview (Section A) which attempts to place the findings in the broader context of current environmental health concerns.



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SUMMARY

Short-term health effects of common ambient air pollutants, particularly photochemical oxidants, were investigated under controlled conditions simulating typical ambient exposures. Volunteer subjects were exposed, in an environmental control chamber providing highly purified background air, to single pollutants or mixtures under conditions of realistic secondary stress (heat and intermittent exercise). Normal men exposed to ozone (O_3) showed respiratory symptoms, pulmonary function decrement, and alterations in red-cell biochemistry. These effects were dose-related, with apparent "threshold" for detectable effect levels as low as 0.2-0.3 ppm in a 2-hr exposure for the most sensitive subjects. Addition of 0.3 ppm nitrogen dioxide (NO_2) and 30 ppm carbon monoxide (CO) did not noticeably enhance adverse effects of O_3 , but addition of 0.37 ppm sulfur dioxide (SO_2) to 0.37 ppm O_3 produced slightly greater effects than did 0.37 ppm O_3 alone. Subjects with asthma or clinical airway hyperactivity appeared to experience more severe effects of O_3 than normals, and subjects chronically exposed to ambient O_3 appeared to be less reactive than those living in non- O_3 -polluted areas. Normal subjects exposed to NO_2 at 1 ppm or 2 ppm showed little clinical or physiological response, but did show changes in red-cell biochemistry at both concentrations. These effects were less than produced by 0.37 ppm O_3 , but significantly greater than produced by heat and exercise stress alone.

Ozone appears to be the most hazardous of common gaseous photochemical pollutants, with respect to short-term human health effects. Further investigation is needed of additive or synergistic effects of pollutants in mixtures, of chronic effects of repeated exposures, and of effects in population groups with preexisting disease or other high-risk characteristics.

SECTION A. INTRODUCTION AND OVERVIEW

1. Goals

The basic purpose of this project has been to provide part of the scientific data base needed for establishment of ambient air quality standards for the protection of public health, as required by the Clean Air Act of 1970. Since overly stringent controls on pollutant emissions are likely to result in economic harm, just as insufficiently stringent controls are likely to result in harm to human health, it is vitally important that air quality standards for any given pollutant be based on a careful and realistic appraisal of the hazards associated with that pollutant. We sought to determine, for specific pollutants found in photochemical smog, whether or not doses attainable in severe pollution episodes produced detectable short-term adverse health effects in normal adults. A broad range of tests was employed to maximize the probability of detecting any adverse effects which might occur. Chronic effects of often-repeated exposures were not considered; therefore, the results have no direct bearing on questions of public-health risks from chronic exposures.

2. Experimental Design and Methods

The basic experimental approach, facilities, and physiological test procedures are described in detail in Section B, Part 2. Biochemical test procedures are described in Section B, Part 1. In general, a volunteer subject is exposed to purified air to which a carefully controlled amount of a given pollutant has been added, under conditions simulating as closely as possible an ambient exposure to the same pollutant. Following the exposure, health-effects tests are performed. Results are compared with those obtained in a control experiment--an identical exposure to purified air with no pollutant added. Since inhaled pollutants first come in contact with the respiratory tract, sensitive respiratory physiological tests are the logical choice as sensitive indicators of pollutant effects. Pollutant molecules or their reaction products which successfully pass through the respiratory tract enter the circulation, thus blood biochemical variables may also be expected to be sensitive indicators. In studies of oxidant pollutants, biochemical investigations are logically directed to the pentose-pathway enzymes of the red cell, which are important in maintaining its correct redox potential, as well as to the cell membrane--the cell's first line of defense against toxic substances.

Exposure conditions in most instances were chosen to simulate weather conditions typically prevailing during photochemical pollution episodes in Southern California--temperature 31°C (88°F) and relative humidity 35 percent. These conditions are also realistic with respect to photochemical episodes in other parts of the United States, except that humidity may be somewhat higher in other areas. Exposures were also performed under "normal room temperature" conditions--21°C (70°F) and 50 percent relative humidity--when appropriate.

Calibration of pollutant monitors is described here since it is not covered in detail in Section B. Chemiluminescent ozone (O_3) monitors are calibrated by the EPA 1 percent neutral buffered potassium iodide method (1) using O_3 -containing purified air at 35 percent relative humidity as the sample atmosphere. This calibration is similar to that previously used by the State of California, which employed 2 percent buffered potassium iodide (2). The current California standard is based on an ultraviolet absorption method which gives readings about 20 percent lower than given by the above methods. (3) A commercial ultraviolet instrument with calibration traceable to the California standard (DASIBI Environmental Corp. 1003-AH) is currently used as a calibration check; however, concentrations are reported in terms of the EPA method. Nitrogen dioxide calibrations are performed with a National Bureau of Standards permeation tube whose output is diluted with dry nitrogen, then with air filtered by silica gel, activated carbon, and molecular sieves. Sulfur dioxide calibrations are performed similarly with a Metronics permeation tube. Carbon monoxide calibrations are performed with commercial calibrating gases (Scott Laboratories); two or more gas cylinders are compared for consistency as a check on reliability.

3. Results

Detailed results are given in Section B. The following is a brief description of work performed, approximately in chronological order.

Initial exposure studies employed 1 ppm NO₂ (approximating the highest ambient concentration observed in the Los Angeles area). Five staff members served as subjects in pilot studies. No symptoms or adverse clinical findings were produced in this group by exposure for 2 hr or longer. One individual appeared to develop frequency dependence of dynamic lung compliance with exposure, but this finding could not be reproduced in later studies. No other meaningful pulmonary function changes were observed. In further investigation of NO₂ effects, 15 healthy male pre-professional students were exposed to 1 ppm for 2 hr on each of two successive days. No meaningful pulmonary function changes were found, nor were any found in a smaller group exposed to 2 ppm. Blood biochemical changes suggestive of an oxidant challenge were found at both concentrations, however. To test the possibility that heat and/or exercise stress alone could account for these changes, another group was exposed to pure air only on three successive days. Biochemical measures were essentially unaffected by this exposure.

Initial studies of O₃ employed exposures to 0.50 ppm—experienced occasionally in Los Angeles and rarely, if at all, elsewhere. The question whether effects could be shown under the worst possible realistic exposure conditions was addressed by exposing four staff members for 4 hr (twice the typical duration of an ambient exposure) on two successive days in each of three successive weeks. To test whether coexisting pollutants might modify O₃ effects, 0.3 ppm NO₂ was added to the exposure atmosphere the second week and 30 ppm CO plus 0.3 ppm NO₂ the third week. Total O₃ dose received in the three-week protocol was estimated to equal that received in an entire summer season of ambient exposures. The four initial subjects completed this protocol without showing detectable clinical or physiological effects, although pronounced oxidative changes in biochemical parameters were seen beginning with the first exposure to O₃ alone.

The relative lack of response of the initial subjects prompted us to repeat the same experiment using subjects suspected of being "hyperreactive" to inhaled irritants. These four people had normal baseline pulmonary function but had either mild asthma or a history of respiratory symptoms subjectively associated with smog exposure. All four subjects became ill and showed substantial pulmonary function changes with the first O₃ exposure, thus it was impossible to complete the planned experimental protocol. Subsequent studies were conducted at 0.25 ppm O₃ and 0.37 ppm O₃ for two or four hours and at 0.50 ppm O₃ for 2 hrs, and included both "normal" and "hyperreactive" subjects. No substantial reactions to 0.25 ppm were found, but some "hyperreactors" experienced effects at 0.37 ppm, as did most "hyperreactors" and some "normals" at 0.50 ppm.

At the highest concentration, effects were clearly cumulative in some individuals exposed on two successive days, with substantial increases in symptoms and function decrement occurring on the second day. This finding is important in that pollution episodes often recur on several successive days. Biochemical, physiological, and symptom data showed dose-response relationships with apparent "thresholds" near 0.25 ppm.

In comparing our results with those reported by Bates's group in Canada (4,5), we observed that the Canadian subjects' response to O_3 appeared about twice as severe at a given exposure concentration. Bates and Hazucha also reported that a mixture of 0.37 ppm O_3 plus 0.37 ppm SO_2 produced far more severe physiological responses than did 0.37 ppm O_3 alone (6). To evaluate the significance of the Canadian findings, we undertook a series of comparative studies. When subjects previously studied in Canada were reexposed to O_3 in this laboratory, their responses were similar to those previously seen in Canada and the more reactive individuals showed more severe effects than even the "hyper-reactors" who were Los Angeles area residents. Similar results were obtained in a subsequent study of student volunteers--Los Angeles residents were less reactive on the average than nonresidents, even though resident and nonresident groups were well matched in other characteristics. Thus it appears likely that adaptation which modifies short-term O_3 effects develops in Los Angeles residents. In studies of O_3/SO_2 mixtures, Los Angeles residents failed to show the marked synergistic effect seen by Hazucha and Bates, but those studied did show slightly increased effects with the mixture relative to O_3 alone. Canadian subjects studied in this laboratory showed responses less severe on the average than they had previously showed in Canada, but more severe than were seen in Los Angeles residents. Individual variability was considerable, making firm conclusions difficult. One possible explanation of these findings which remains to be tested is that accompanying particulate pollutants contributed to the O_3/SO_2 effect in the Canadian studies, in which highly efficient air filtration such as used in our studies was not available.

4. Conclusions

a. Ozone

Ozone exposure produces loss in pulmonary function and respiratory symptoms severe enough to impair performance of normal tasks at levels easily attainable in Los Angeles area smog episodes and probably occasionally attainable elsewhere. Blood biochemical changes also occur; these cannot as yet be shown to be correlated with physiological or clinical adverse effects, but must be considered harmful in the absence of evidence to the contrary. Physiological and clinical effects of exposure are generally reversed within 24 hrs after exposure ceases, but biochemical variables appear to require longer to return to baseline levels. While in the population studied, the average "threshold" of detectable effect appeared to be 0.25 ppm O_3 or higher, effects might well be found at lower concentrations in high-risk groups such as the young, the old, and persons with preexisting cardiopulmonary disease. Further investigation of high-risk groups will be needed to improve the scientific data base for air-quality standards. While adaptation probably operates to reduce the acute effects of O_3 exposure in individuals exposed repeatedly, this should not be considered a justification for relaxing O_3 air quality standards, since the adaptive process might have other harmful effects and might not operate satisfactorily in some individuals.

b. Ozone in Mixtures

When realistic concentrations of NO_2 and CO were added to O_3 , no additional effects were observed. This experiment was done only with O_3 concentrations which produced little or no clinical or physiological effect in the individuals studied (0.25 ppm in "hyperreactors", 0.50 ppm in "normals"). The possibility that coexisting NO_2 might exacerbate effects of O_3 exposures above the "threshold" level has not been investigated. Adding SO_2 to O_3 concentrations near the "threshold" level did appear to produce modest increases in exposure effects. Large numbers of sulfate particles were also formed in the mixture. Further investigation of the health-effects role of particulates in pollutant mixtures of this kind will be necessary before health hazards of ambient mixtures can be assessed reliably.

c. Nitrogen Dioxide

Exposures to 1 ppm or 2 ppm NO_2 produced no meaningful physiological changes in the population studied. A minority of the study population experienced mild respiratory symptoms possibly, but not certainly, attributable to exposure. Blood biochemical changes were seen at both concentrations. These were not clearly dose-related, and a threshold of detectable effect has not been determined. While the physiological and clinical findings suggest that NO_2 is far less hazardous than O_3

at ambient concentrations, the biochemical findings must, in the absence of contrary evidence, be considered as indicating an adverse health effect at concentrations of 1 ppm and possibly lower. Furthermore, Orehek et al. (7), recently reported that NO₂ produced an increased reactivity to bronchoconstrictor challenge in some asthmatics at concentrations as low as 0.1 ppm--a level commonly experienced in most urban areas. The report of that study does not make clear whether control of the experimental environment was sufficiently rigorous to assure that the observed effects were entirely due to NO₂; however, the work at least raises the possibility that some susceptible individuals may be adversely affected even by relatively low ambient pollutant levels.

d. General Comments

In the course of this project and concurrent studies elsewhere, there has developed considerable understanding of the short-term health effects of individual gaseous pollutants in relatively healthy people. Most needed now is information on health effects of particulate pollutants and mixtures of gaseous and particulate pollutants, both in healthy and in diseased or hypersusceptible populations. Since the possible combinations of ambient pollutants and resulting health-effects interactions are almost infinite, controlled-exposure studies like those reported here can hope to provide only a part of the necessary health-effects data base. The rest must be provided by studies of people exposed to actual ambient pollution, in which both health-effect variables and environmental variables are quantitated as comprehensively and carefully as possible in order to document relationships between health and specific pollutants or mixtures. In addition to large-scale epidemiologic investigations, smaller studies should be considered in which panels of clinically well-characterized individuals are placed in a given well-monitored polluted environment and examined for short-term health effects attributable to that environment. This approach should help bridge the gap between controlled-environment laboratory studies, in which results may be difficult to relate to real-life health hazards, and epidemiologic studies, in which the reliability and sensitivity of the health-effects testing is often inadequate to detect or quantitate the health hazard of concern. Possible chronic effects of repeated pollutant exposure are an additional serious research problem which must be approached mostly by epidemiologic and animal toxicology studies.

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SECTION B

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Ozone and Human Blood

Ramon D. Buckley, PhD; Jack D. Hackney, MD; Kenneth Clark; Clara Posin

Statistically significant changes ($P < 0.05$) were observed in erythrocytes (RBC) and sera of young adult human males following a single short-term exposure to 0.50 ppm ozone (O_3) for 2 1/2 hours.

The RBC membrane fragility, glucose-6-phosphate dehydrogenase (G-6-PDH) and lactate dehydrogenase (LDH) enzyme activities were increased, while RBC acetylcholinesterase (AcChase) activity and reduced glutathione (GSH) levels were decreased. The RBC glutathione reductase (GSSRase) activities were not significantly altered. Serum GSSRase activity, however, was significantly decreased while serum vitamin E, and lipid peroxidation levels were significantly increased. These alterations tend to disappear gradually, but were still detectable two weeks following exposure.

The toxicity of inhaled ozone (O_3) is well known and the presence of the oxidant in many urban and industrial environments has made the study of the biological effects of inhaled O_3 important.¹ Recent studies have been directed toward the clari-

fication of the biochemical changes that occur in tissues following O_3 exposure. Radiomimetic changes were described² as well as the oxidation of unsaturated fatty acids,³ and oxidation of biologically active reducing substances such as reduced sulfhydryl groups and the cofactors NADH and NADPH.⁴ Sufficient knowledge is now available to allow speculation about the importance of some of the observed changes in the development of acute or chronic pulmonary disease, or in the development of O_3 tolerance. Ozone is known to produce a dose-related reaction in *in vitro* cell cultures extending from mild metabolic suppression at low levels to cell death at high levels.⁵ It is natural that the target organ for O_3 toxicity studies is the lung, and much of our knowledge derives from studies of effects of oxidants in this organ. The results point to loss of reduced sulfhydryl groups and reduction of activities of sulfhydryl-containing enzymes, while the pentose and glycolytic pathway activity levels are increased.⁶⁻⁸ Oxidant effects of O_3 inhalation have also been shown to occur in the blood of rodents following high, but sublethal levels of the irritant, and changes in erythrocyte (RBC) and sera also suggest that oxidant-induced alterations have taken place beyond the blood-air barrier.^{9,10}

It is not known if O_3 at ambient levels crosses the air-blood barrier in humans or if detectable biochemical changes occur. The present study was undertaken to answer that question.

SUBJECTS AND METHODS

Seven healthy young adult human male volunteers were studied. The same chamber was used for sham control exposures and for exposure to O_3 . The chamber contents are monitored for O_3 , NO_x , CO, hydrocarbons, and particles. Pollutant levels were essentially zero during sham exposures, and chamber background particle levels (0.5μ to 5μ) were less than 20,000/cu ft when empty, and less than 120,000/cu ft during subject exercise. Subjects performed identical exercise and pulmonary function tests during a sham control period and while O_3 was being administered. During exposure, a 0.5 ppm atmosphere of O_3 , produced by a silent-arc generator, was added to the chamber air supply. Chamber temperature was maintained at 30 C and relative humidity at 35% to simulate a typical summer day in Los Angeles.

Venous blood samples were collected in heparinized and unheparinized tubes immediately after the sham and the O_3 exposure. Heparinized blood was stored on ice until the erythrocyte (RBC) studies could be completed. The unheparinized blood was allowed to remain at 2 to 4 C for four to six hours until the serum could be removed.

Experiments were planned to detect blood tissue oxidation after O_3 inhalation since this has been shown to be the principal effect of high levels of the irritant on lung and blood tissues of experimental animals. The methods are essentially the same as described in the following references with only minor modifications. The RBC membrane fragility was measured by determining the degree of hemolysis in the presence of H_2O_2 .¹¹ Activities of RBC enzymes glucose-6-phosphate dehydrogenase (G6PDH),¹² lactate dehydrogenase (LDH),¹³ glutathione reductase (GSSRase),¹⁴ and acetylcholinesterase (Ac-

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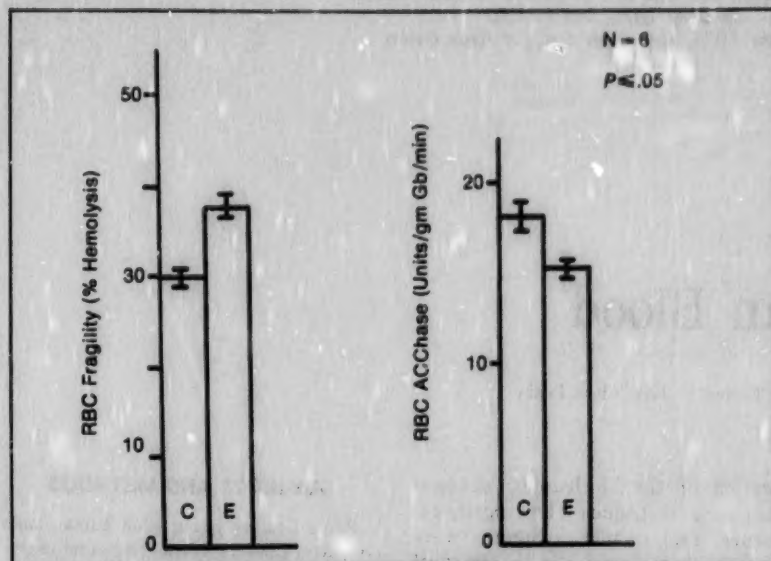
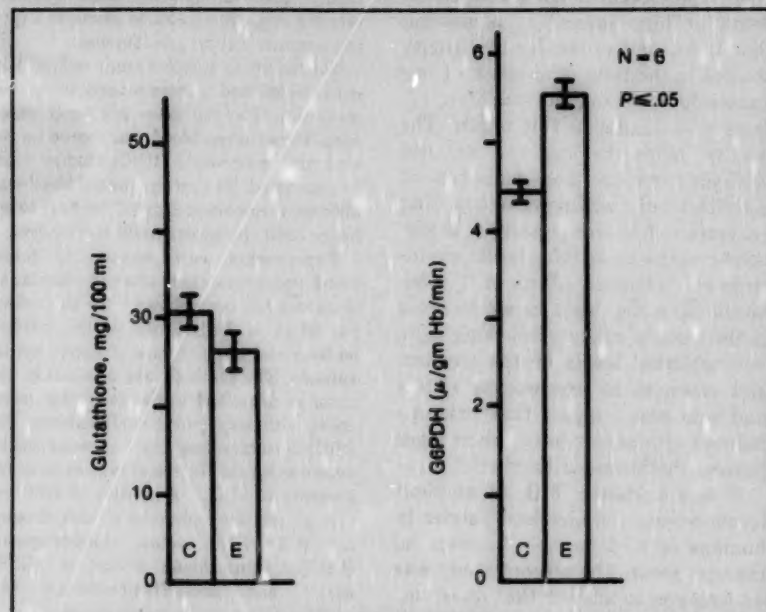


Fig 1.—Human RBC response to inhaled ozone (0.5 ppm $\times$ 2 hr 45 min). Fragility was measured as percent hemolysis in 2% hydrogen peroxide and incubated for one hour at pH 7.4 in Krebs-Ringer bicarbonate buffer. Acetylcholinesterase was measured at pH 8.0 in 0.1 M phosphate buffer employing acetylthiocholine as substrate. Activity is expressed as mM/ml of blood/min.

Fig 2.—Human RBC response to ozone (0.5 ppm $\times$ 2 hr 45 min). Glutathione assay detects soluble GSH employing 5, 5' dithiobis (2-nitrobenzoic acid) as coupling reagent.



Chase),¹⁸ were measured as well as RBC glutathione levels.¹⁹ Serum levels of vitamin E,^{17,20} and lipid peroxides²⁰ were determined as well as activity levels of the serum enzyme glutathione reductase.²⁰

Each experimental subject served as his own control and paired-group analyses were performed. The small "Student's" *t*-test was used to test the null hypothesis, with the critical level at $P < .05$.

RESULTS

Data in Fig 1 to 4 are means and standard errors of the means, of paired-group analyses of seven experimental subjects.

The evidence indicating O_3 -induced changes in the RBC membrane is shown in Fig 1. The single O_3 exposure resulted in a significant increase ($P < .001$) in RBC fragility to H_2O_2 , while the activity of the membrane-bound enzyme AcChase was decreased ($P < .001$). Figure 2 shows that the activity of G6PDH was significantly increased ($P < .001$) while RBC levels of GSH were decreased ($P < .01$). Ozone inhalation also stimulated an increase in LDH activity ($P < .001$) although RBC GSSRase activities were not altered (Fig 3). Serum GSSRase activity levels are shown in Fig 4 to be significantly decreased ($P < .05$). Vitamin E levels were also increased ($P < .025$) at the same time that increased oxidation of unsaturated fatty acids ($P < .025$) was observed.

COMMENT

The very high oxidation potential of O_3 has led research workers from the beginning to suspect that the major damage from inhalation of this irritant resulted from oxidation of labile components in biological systems to produce structural or biochemical lesions.²¹ More recent work has verified that inhaled O_3 causes oxidation of components of rodent lung.²² Other studies have shown that changes in the rate of tissue metabolism also accompany O_3 -induced changes in the oxidation states of lung tissue components.²³

This study arose from our need to seek evidence of changes in human tissues due to inhalation of ambient levels of O_3 , and was encouraged by evidence from past experiments that showed that significant alterations in

the blood tissue of rodents did occur as a result of inhalation of oxidants, although the levels were generally much higher than ambient levels anticipated during a smoggy day. Experiments performed by others,¹⁰ and in our laboratories,^{24,25} have shown that inhalation of high levels of O_3 by rodents results in oxidation changes in blood similar to those detected in lung. Considerable question arose about possible metabolic changes in blood following low-level O_3 inhalation, since it is known that the efficiency of the upper airway in the removal of O_3 is quite high, and that the efficiency increases as the levels of inhaled O_3 decrease.²⁶ The results indicate that under the conditions of the experiment a single exposure to 0.5 ppm for 24 hours is above the threshold level.

The observed changes suggest that oxidation is the initial event, and that additional changes occur as a result of the system's attempt to compensate for the changes in blood tissue redox potentials. Reduced glutathione, considered to be an important biological antioxidant, is significantly decreased by O_3 inhalation. The RBC enzyme acetylcholinesterase (AChase), containing a -SH group essential for its activity is also depressed. The increase in the presence of peroxidized lipids and increase in vitamin E in the sera also suggest that oxidation is responsible for the primary O_3 response. Glutathione reductase (GSSRase) activities showed no change in RBCs while the serum enzyme activities were decreased. We have no explanation for this observation except to suggest that the RBC enzyme may be protected by its site within the cell.

The increases in glucose-6-phosphate dehydrogenase (G6PDH) and lactate dehydrogenase (LDH) activities suggest that RBC metabolism is stimulated by O_3 . The increase in G6PDH activity may also be related to the adaptation phenomenon described by others.²⁷ One of the possible pathways by which tissue levels of GSH are maintained is schematically represented in Fig 5. An increase in G6PDH activity could provide reduced cofactor essential for the

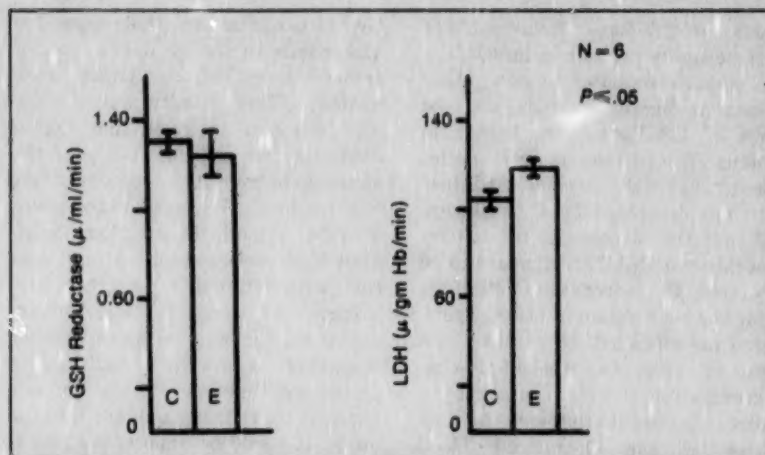


Fig 3.—Human RBC response to ozone (0.5 ppm $\times$ 2 hr 45 min). Glutathione reductase activity was expressed as international units/ml of blood/min. Oxidized glutathione was used as substrate. Lactate dehydrogenase activity was measured by following the disappearance of NADH at 340 nm and was expressed as international units/gm hemoglobin/min.

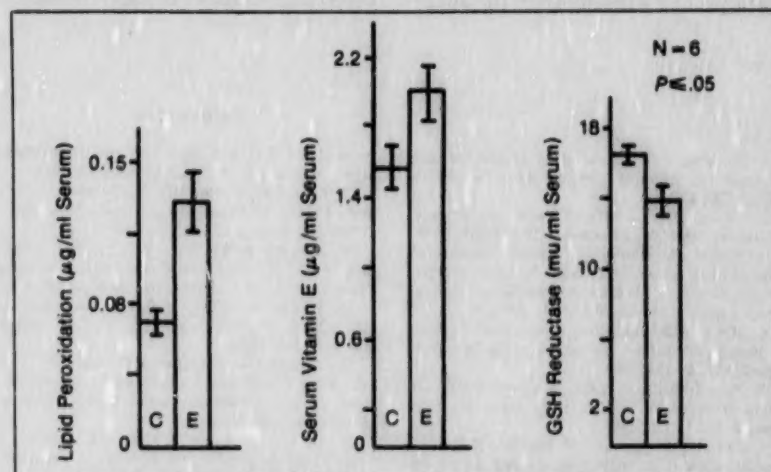
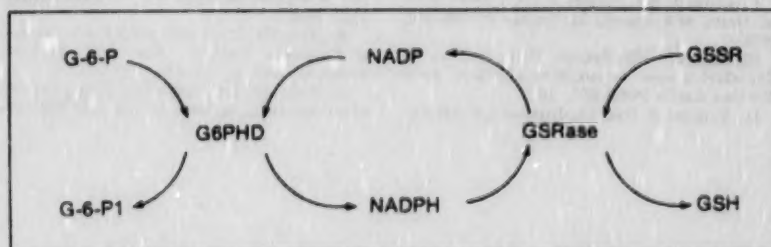


Fig 4.—Human inhalation of ozone, serum. Lipid peroxidation is expressed as micrograms of malonaldehyde per milliliter of serum. Vitamin E is expressed as microgram of α -tocopherol per milliliter of serum. Glutathione (GSH) reductase activity was expressed as millunits (mu) per milliliter of serum per minute.

Fig 5.—Possible metabolic pathway by which normal erythrocyte redox levels could be maintained.



function of GSSRase. The amounts of GSH normally present in human tissues is small compared to many other species of higher animals but the levels of GSSRase were higher in humans (RBCs) than in any species tested.²⁷ The RBC levels of GSSRase were not decreased by O₃ exposure, and thus the mechanism for the regeneration of NADPH appears to be unaltered. The increase in G6PDH activity has been shown in other experiments (unpublished) to persist for a period of at least two weeks following O₃ stimulation. It will be important in future experiments to follow the time course of the O₃-induced blood changes in order to determine the length of time they persist and if they are related to the adaptation phenomenon observed in laboratory animals.

The increase in serum vitamin E levels probably results from the adap-

tive response of the whole organism and results in the availability of increased levels of circulating antioxidants. This mobilization of vitamin E again suggests that O₃, or oxidizing free-radical, does pass the air-blood barrier at the comparatively low levels used in the experiment. Whether vitamin E acts alone to inhibit lipid peroxidation,²⁸ acts in concert with vitamin A or other substances to protect tissue from oxidation,²⁹ or functions in some other way, there is now little doubt of its protective function^{24,25,30} in animals exposed to a strong oxidant such as ozone. It would be important in a future experiment to determine if ingestion of relatively large amounts of vitamin E would protect humans against the biochemical effects of O₃.

Future experiments will determine the level of inhaled O₃ below which no observable changes occur in humans.

Knowledge of these "threshold" levels will be useful in determining the acceptable and permissible atmospheric standards. It will also be important to establish the length of time required for changes to disappear, as well as to determine if successive exposures produce cumulative effects. A very wide range of subjective reactions to oxidizing air pollution is expressed by people living in cities where this type of smog is severe. It will be important to seek possible correlations between the degree of subjective irritative response and biochemical change, to help determine if differences exist in people's capacity to resist O₃.

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Experimental Studies on Human Health Effects of Air Pollutants

I. Design Considerations

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Because of the possible threat to public health posed by photochemical air pollution, a need exists for experimental studies of short-term respiratory effects of air pollutant exposure in humans. Such studies require rigorous control and comprehensive documentation of the experimental air environment and exposure conditions to ensure that results are both reliable and relevant to public health questions.

In addition to biochemical, behavioral, and clinical evaluations, comprehensive pulmonary testing is required to assure that effects at different levels of the respiratory tract are detected. An experimental design based on these principles is described. Studies using this design have shown a wide range of sensitivity to the pollutant ozone and important adverse health effects in sensitive individuals under exposure conditions similar to those experienced during ambient pollution episodes.

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Photochemical smog is a complex mixture of substances, including powerful oxidizing agents such as ozone (O_3), nitrogen dioxide (NO_2), and organic peroxides. It is formed by the action of atmospheric oxygen and sunlight on effluent gases, particularly hydrocarbons and nitric oxide (NO), emitted as a result of automotive and industrial fuel combustion.¹ The respiratory and other health effects of exposure of humans to photochemical smog have not been well documented. The Los Angeles area is most often associated with such exposures, but they are by no means limited to this region. Substantial photochemical oxidant concentrations have been reported in Canada² and in Europe,³ and can probably occur in most areas where there are concentrations of automobile traffic or fuel-burning industry when sunlight is strong and winds are too light to disperse effluent gases. Thus, photochemical smog and other air pollutants present a widespread potential public health problem. The appropriate government agencies have responded by setting air quality standards intended to protect the population from dangerous levels of exposure. Most existing standards, however, are based on limited scien-

tific information on the health effects of pollutants.⁴ Legal challenges to the standards are also frequent because the economic and social costs of conforming may be high. It is thus apparent that there is a need for comprehensive experimental studies on the health effects of exposure to oxidants and other air pollutants. Such studies must be controlled and documented as rigorously as possible to ensure reproducibility and to withstand legal challenges. Although numerous animal studies exist and others are in progress, at present they cannot be quantitatively related to human health. Epidemiologic studies can be useful, but are limited by cost, dose-range available, presence of interfering pollutant substances, and problems caused by many uncontrolled variables.

Human experimental studies of the health effects of oxidant air pollutants can be accomplished at fixed concentrations in the absence of interfering pollutants, under well-controlled environmental conditions and with a well-characterized subject group of limited size. Studies of the health effects of well-specified ambient air are also possible. A survey of the environmental control and monitoring technology used in previous

Table 1.—Chamber Environmental Control Factors

Measure	Design Specification		Actual Performance	
	Ambient*	Chamber	Ambient	Chamber†
Temperature, F	25-110	Within ± 1 , range 14-110	25-110	Within ± 1 , range 15-130
Relative Humidity	10%-100%	Within $\pm 4\%$, range 10%-100%	10%-100%	Within $\pm 5\%$, range 20%-100%
Total No.‡ particles per cubic foot	$\geq 10^4$	$< 10^3$	$\geq 10^4$	200-400§ $2 \times 10^3 - 10^4$ $2 \times 10^4 - 10^5$
CO, ppm	30	2	20#	1
NO, ppm	2	.01	1#	0.02
NO ₂ , ppm	1	.01	0.7#	<0.01
O ₃ , ppm	0.7	.01	0.4#	<0.01
SO ₂ , ppm	1	.05	1**	0.01**
Hydrocarbons, ppm	30	5	100**	5**

* Extreme ambient conditions experienced in Los Angeles area.

† Over at least a six-hour period.

‡ Particles with diameter $\geq 0.5 \mu\text{m}$.

§ No one in chamber.

|| One to four subjects at rest.

|| One to four subjects exercising.

Maximum concentration typically found outside of the environment at control facility.

** When deliberately challenged at or above the ambient level.

experimental studies^{1,2} indicates that many limitations existed. More rigidly controlled studies are thus required to extend previous work.

Bates et al and Hazucha et al³⁻⁵ have discussed some of the problems encountered in the design and execution of experimental studies on humans exposed to air pollutants, and have described an appropriate experimental protocol. Despite some limitations in environmental assessment and control, they have verified impairment in pulmonary function in subjects exposed to ozone concentrations equal to or less than those encountered in severe photochemical smog episodes. This communication is intended to report the operational approach for a new series of studies, drawing on the experience of Bates and others, and using current technology to provide more rigorous experimental control and more comprehensive information than was obtainable previously on the effects of human exposure to pollutants, singly and in combination. A unique environmental control facility combined with an interdisciplinary research team provides the capability to conduct this research.

The basic design of studies of pollutant exposure should seek to maximize information relevant to public health. The tests for effects of smog must be reliable and sensitive, the experimental atmospheric environment must be

rigorously controlled and; equally important, the manner in which subjects are exposed to the experimental environment must realistically simulate actual air pollution exposures if results are to be relevant. These constraints impose complications in experimental design and necessitate focusing attention on several distinct problems: environmental control, pollutant generation, environmental monitoring, physiological testing, and evaluation of symptoms and clinical observations. The following sections describe approaches to each of these problems.

FACILITIES Environmental Chamber

Studies are performed in the Rancho Los Amigos Clinical Environmental Stress Testing Laboratory. This facility consists of a stainless steel-sheathed controlled environment chamber, approximately 28 sq m in area, accessible through a double-door lock compartment that contains lavatory facilities and through which air is exhausted. The main chamber contains physiological test equipment and can hold five subjects at the same time. Data recording and monitoring equipment are located outside the chamber in an adjacent laboratory area. Air flows in an approximately laminar manner through the main chamber from ceiling to floor at a rate that provides a complete change of air

every five minutes, and is then exhausted without recirculation. The air is highly purified and can be adjusted to simulate a wide range of meteorological conditions (Table 1). The air purification unit (Mine Safety Appliances, Inc.) contains high-efficiency particulate filters, a catalytic oxidation unit for conversion of carbon monoxide and hydrocarbons to carbon dioxide, and chemical filters containing activated charcoal and aluminum oxide pellets impregnated with potassium permanganate (Purafil, Inc.). The air-conditioning unit consists of refrigerant coils, heaters, and steam injectors, controlled automatically to maintain desired levels of temperature and humidity.

Pollutant Generation

Each pollutant gas is introduced through its own stainless steel inlet line into the inlet duct for purified air. Complete mixing occurs before the air reaches the main chamber, producing uniform concentrations throughout the chamber (within 5% of the mean value). Each inlet line incorporates a safety device that stops generation if electric power fails. Carbon monoxide, nitric oxide, nitrogen dioxide, sulfur dioxide, and ozone have been studied. The CO may be introduced from a cylinder of pure gas with appropriate flowmeter. The NO and SO₂ are similarly introduced from cylinders in the form of nitrogen-diluted mixtures.

The NO_x is introduced by bubbling nitrogen gas through a cylinder of liquid N_2O , and metering the resulting $\text{N}_2\text{-NO}_x$ mixture through a specially designed flow control apparatus. Ozone is generated from dried and filtered air using a high-voltage discharge ozonator (Welsbach T-408). No contaminating nitrogen oxides are produced when this technique is employed. All pollutants can be generated in concentration ranges realistically simulating ambient conditions and concentrations can be controlled to within 10% of the expected value.

Environmental Monitoring

The chamber atmosphere is monitored continuously utilizing instruments and techniques equivalent to those used in ambient air monitoring networks. Instruments are calibrated as recommended by the California Department of Public Health and the California Air Resources Board.² Cross comparisons of ozone monitoring techniques are made with analytical laboratories of the latter agency. When it is possible, two different monitoring instruments are used for each gaseous pollutant under study. Ozone and nitrogen oxides are monitored using chemiluminescent analyzers (Models 612 and 642, REM Scientific), which provide fast response and freedom from interference by other pollutants. Total oxidants (ie, ozone) and nitrogen oxides are also monitored by the neutral buffered potassium iodide solution and Saltzman reagent methods, using continuous-flow colorimetric analyzers (Beckman K-76). Carbon monoxide is monitored by a nondispersive infrared analyzer (Mine Safety Appliances) and by an oxidative electrochemical analyzer (Model 2100, Energetics Science, Inc.). Sulfur dioxide is monitored using a flame photometric analyzer (Melo SA-160). A light-scattering, single-particle counter (Royco Instruments, Model 225) monitors particulates in five subranges between 0.5μ and 10μ in diameter.

METHODS

Physiological Testing

An initial target of any air pollutant challenge is the respiratory tract,

which is thus the center of attention in tests of effects of exposure. Other areas of interest include hematology, blood enzyme biochemistry, and psychomotor performance. Insult by pollutants can be manifested at various sites in the respiratory system. Bronchoconstriction in the large airways, maldistribution of ventilation due to hypersecretion in small airways, constriction of alveolar units, and impairment of diffusion as a result of edema are possible effects. A variety of pulmonary tests is required to examine the various possibilities; those employed in this study are described below.

Flow-Volume Curves.—These are recorded using a low-resistance spirometer (Electro-Med 780). Partial and maximum forced expiratory maneuvers are performed. Partial forced expirations are initiated at 65% of vital capacity. These tests may be affected more by mild bronchoconstriction than are full-vital capacity forced expirations.¹⁰ The functions measured are forced vital capacity (FVC), one-second forced expiratory volume ($\text{FEV}_{0.1}$), peak expiratory flow rate ($\dot{V}_{\text{max}}$), and flow rates at 50% and 25% FVC ($\dot{V}_{0.5}$, $\dot{V}_{0.25}$) for partial and maximum flow-volume curves. These measurements give an easily obtained, relatively reproducible evaluation of overall pulmonary mechanical performance, but provide little information on the mechanisms responsible for any observed changes.

Airway Resistance (R_{aw}) and Thoracic Gas Volume (TGV).—These are determined in a whole-body plethysmograph using the method of DuBois et al.^{11,12} The measurement of R_{aw} is more sensitive to bronchoconstriction than maximum-flow measurements, but the test is more difficult to perform and less stable. These problems similarly affect the measurement of TGV which, however, may be useful for detecting gas trapped as a consequence of airways dysfunction (in combination with a gas-dilution lung-volume determination).

Total Respiratory Resistance (R_{t}).—This is determined by the forced oscillation technique.¹³ The method of Goldman¹⁴ is used to eliminate the need to achieve or simulate resonance.

To eliminate the phase shift introduced by Fleisch pneumotachograph at higher frequencies,¹⁵ phase compensation is used to ensure correct relationships of the flow and pressure signals. Resistance is measured at pressure perturbation frequencies of 3, 6, 9, and 12 hertz. This measurement is affected by changes in upper-airway configuration, which may complicate detection of changes in pulmonary airways per se. The method is believed to be capable of detecting asynchronous mechanical behavior (unequal regional ventilatory time constants) as predicted by Otis et al.,¹⁶ which otherwise can be documented only by the considerably more difficult measurement of dynamic lung compliance.

Closing Volume (CV).—This is determined by the single-breath nitrogen washout method¹⁷ using a linear nitrogen analyzer (Med-Science 505). This test is believed to be sensitive to changes in small airways in dependent lung regions. It determines the lung volume at which closure of a great number of small airways presumably occurs, and also provides an estimate of residual volume (RV) and total lung capacity (TLC) through the expired nitrogen concentration¹⁸ and an estimate of the uniformity of ventilation distribution through the slope of the alveolar plateau.¹⁹

Static and Dynamic Lung Compliance (C_{st} , C_{dyn}).—These are measured from recordings of transpulmonary pressure and respiratory flow and volume. Transpulmonary pressure is measured by the esophageal balloon method of Milic-Emili et al.²⁰ Flow at the mouth is measured by a pneumotachograph (Fleisch) and volume by a spirometer (Electro-Med 780). Adequate dynamic response of the system has been verified at frequencies up to 100 breaths/min. Dynamic compliance in the tidal range is measured at normal breathing frequency and at 20, 40, 60, 80, and 100 breaths/min with tidal volume monitored and kept constant at 0.75 liter. Static compliance is measured by closing a mouth shutter intermittently during an inspiration from functional residual capacity (FRC) to TLC, followed by an expiration to RV. Static compliance determi-

nations are preceded by inspirations to TLC to give a consistent volume history.

Compliance measurements are indispensable for delineation of changes in the mechanical characteristics of the lung, particularly the development of unequal time constants. Unfortunately, the measurements are somewhat unstable and require considerable effort. In this study these tests are performed only on a subgroup of subjects selected for motivation and performance.

Pulmonary Diffusing Capacity (DL_{CO}).—This is determined by the single-breath carbon monoxide method.¹¹ In the calculation of DL_{CO} correction is made for back pressure of CO due to substantial levels of carbon monoxide hemoglobin in the blood of some subjects. Reproducibility of this test is poor under conditions of this study (heat and intermittent exercise), but the test offers the possibility of detecting changes in the blood-air interface (such as alveolar edema) that might otherwise go undetected.

Oxygen Consumption.—This is measured at rest and during exercise on a constant-load bicycle ergometer (Model 844, Quinton Instruments) at a level yielding 65% of predicted maximum oxygen consumption.¹² Expired air is collected in meteorological balloons and emptied into a spirometer (Collins 120-liter) to determine total expired volume. Gas samples are analyzed for oxygen using a paramagnetic analyzer (Beckman E-2) and for carbon dioxide using a gas chromatograph (Beckman GC-M). Oxygen consumptions are calculated after the method of Consolazio et al.¹³ A telemetry system (Spacelabs, Inc.) records an exercise electrocardiogram during this test.

Carboxyhemoglobin Concentration (COHb).—This is estimated from CO concentration in alveolar air after a 20-second breathhold, using the method of Jones and co-workers.¹⁴ The CO is measured with an electrochemical analyzer (Model 2100, Energetics Science). The test is performed prior to exposure in the chamber to verify that the subject has not received an inordinate ambient pollutant exposure and performed again at the

Table 2.—Experimental Protocol		
Overall Exposure Schedule		
Week 1, O_2 ; week 2, $O_2 + NO_2$; week 3, $O_2 + NO_2 + CO$		
Weekly Schedule		
Monday, Tuesday (Wednesday)*		Control (clean air), 4 subjects
Thursday, Friday		Pollutant exposure, 4 subjects
Daily Schedule		
Subject No.	Begin Exposure	Begin Test Cycle
1	0700 hr	0645 hr
2	0800	0945
3	0900	1045
4	1000	1145
Individual Subject Schedule		
Time, hr, min	Procedure	
-0:01	COHb	
0:00	Enter chamber, exercise first 15 min of each half hour	
1:45	Last rest period; psychomotor performance test*	
2:00	Respiratory resistance (forced oscillation)	
2:05	Flow: volume maneuvers	
2:10	Closing volume	
2:15	Body plethysmography*	
2:22	Lung compliance*	
2:40	Exercise testing*	
2:58	COHb	
3:00	DL_{CO} *	
3:15	Exit chamber, venous blood sample	
3:20	Physician interview and examination	

* Deleted in abbreviated test protocol.

conclusion of the chamber exposure period.

Symptomatology.—Immediately after exposure each subject is questioned concerning symptoms by the project physician according to a standard questionnaire. Subjects also keep a standard record of symptoms during and after exposure. Caution is exercised in interpreting symptoms since these are not blind studies (see Experimental Protocol).

Clinical.—An attending physician is present in the chamber area during the study and is able to view its progress by means of closed-circuit television. In addition, heart rate, and ECG are monitored via telemetry. Equipment necessary for treatment of an acute cardiovascular or respiratory emergency is maintained in the chamber area.

Development of substantial chest pain during exercise, cardiac irritability, intractable wheezing, or ECG changes in any subject, constitutes an indication for termination of the study for that subject and a thorough clinical examination, with subsequent treatment, if indicated. In the event untoward symptoms or intercurrent illness occurs and the subject is willing to continue, the attending physician makes the ultimate decision as to

whether or not a subject is to persist in the study.

EXPERIMENTAL PROTOCOL

The exposure protocol has been designed to simulate as realistically as possible the ambient exposure of a person working outdoors on a smoggy summer day. A two-hour exposure period is realistic in that high ambient pollutant concentrations usually persist about that length of time. Intermittent light exercise (sufficient to approximately double minute volume) during exposure gives a realistic level of ventilation (to which pollutant dose is proportional) during work. Elevated temperature is an additional stress factor frequently present during air pollution episodes, and consequently introduced into the experimental situation. The design provides for exposures on successive days, as deleterious effects of exposure may be cumulative. These requirements are incorporated into the protocol in a cost-effective manner that tests several subjects on a given day and requires staggered exposure and testing periods, precluding blind studies or control measurements on the same day. Thus, since two or three sham control runs (exposures to purified air) precede the pollutant exposure

reliable base line values of the measured functions can be obtained.

A test series may be reasonably designed to support or reject the null hypothesis that no effects of pollutant exposure at realistic levels will be detected in subjects. Results supporting or rejecting the null hypothesis are useful in recommending air pollution standards. This approach provides a simple method to test for combined effects of two or more pollutants: a single pollutant is tested initially, a second pollutant is added to the first in the next test cycle. Exposure times may also be increased to simulate two days of pollutant exposure in one day's testing. If no effects are found, even under the "worst case" exposure conditions, valuable information relevant to standard-setting may be obtained. If effects are found at some point, additional studies will be required to determine if they are attributable to a single pollutant, to a combined effect, or to cumulative effects of repeated exposures. If minimal effects are found in "normal" subjects, "hyperreactive" subjects are studied as the next step. These subjects are characterized by a prestudy history of cough, chest discomfort, or wheezing, associated with allergy or exposure to air pollution.

Table 2 describes the detailed experimental protocol incorporating the features described. A shorter protocol, eliminating certain tests (marked with an asterisk), is also used. This protocol retains tests considered relatively simple to perform and likely to detect effects of exposure. It requires less training of subjects and thus allows study of subject groups more representative of larger populations.

Statistical Analysis

Experimental data are subjected to repeated one-way variance analyses. Post hoc comparisons using the Newman-Kuels test²³ are made when significant *F* values are found. For each measurement comparisons are made among controls, first day of exposure, and second day of exposure, for each subject as well as each subject group. A few significant differences due to random variation may be found

because of the number of statistical comparisons being made; therefore, all observed statistically significant changes must be examined critically for physiological importance.

Subjects

Ethical and legal considerations require that the utmost care be exercised in human experimentation. Risk is inherent in this work as well as in other research,²⁴ but can be minimized by taking all reasonable precautions consistent with satisfactory performance of the study.

The investigators serve as the first subjects for each exposure study. Pollutant exposure levels selected never exceed the highest recorded ambient levels. The exposure environment and the subjects are constantly monitored and immediate corrective measures are taken if hazardous conditions are encountered.

Prospective subjects are screened by a physician who is not associated with the study. A standardized psychological evaluation (Minnesota Multiphasic Personality Inventory)²⁵ is administered on physician request. After a full explanation of the procedure, including known risks and discomfort, informed consent is obtained. All subjects receive a small reimbursement for their expenses and services.

RESULTS

In comparison to previous experimental studies,^{2,26} more rigorous control of experimental variables (pollutant concentration, background air purity, temperature, and humidity) was possible in the present study. Also, more comprehensive biological information was sought through use of the present experimental design. Additional measures included detailed tests of lung mechanics as well as biochemical and behavioral studies. During and after exposure, clinical responses were described according to a standard format by both the subject and the project physician. Details of the following subject variables are reported: age, sex, number of non-smokers, number and type of smokers, health status, socioeconomic level, any known unusual ambient pollution ex-

posure history during the experiment—this included estimation of blood carbon monoxide hemoglobin before each run to help screen for unknown excessive exposure.

A combination of air pollutants of known or suspected health importance was selected. Concentrations were chosen on the basis of recorded ambient levels for metropolitan Los Angeles and adjacent areas. Duration of pollution exposure and associated environmental temperature and humidity were also surveyed and considered in the design. The resulting protocol as described in this communication represents an attempt to mimic "worst case" conditions of summer exposure in the area. Four-hour exposure periods were chosen to compress two successive daily episodes of two-hours each. Thus, four hours exposure on two successive exposure days were used to mimic actual pollution episodes of up to four days' duration. Although the goal was to use a combination of pollutant gases, considerations of medical safety directed that effects of the potentially most toxic gas be established before adding others. Because, in the concentrations chosen, O₃ was considered potentially the most toxic, it was tested the first week; NO_x was added the second week and CO the third.

We have conducted a series of experimental studies using this core protocol. Detailed findings on our first eight subjects exposed to ozone at realistic levels, alone and in combination with other pollutants, are described in a second article in this issue.²⁶ The definite symptoms and functional decrement found in some of these subjects indicated that the exposure time should be shortened. Therefore the core protocol was modified to provide a two-hour instead of a four-hour exposure period. Results of studies on additional subjects using the shortened exposure time are presented in the third article.²⁷ Several general observations that are documented in the accompanying articles are of interest and will be described here:

1. At least some lightly exercising individuals develop discomfort and measurable effects when exposed to

realistic concentrations of ozone.

2. Some individuals are not noticeably affected by ozone doses two or three times greater than those at which other individuals experience symptoms and measurable respiratory dysfunction. Thus, if only group comparisons are made, risks to more sensitive individuals may go undetected—a matter of concern in experimental and in epidemiologic studies.

3. A striking result in exposure studies so far conducted is that, generally, pulmonary tests that are simplest to perform are most reliable in demonstrating changes. "Reliability" in this sense means that changes observed after exposure are significant compared to the normal test-

to-test variability under control conditions. These tests include FVC, FEV₁, V₅₀, Rt, and the slope of the alveolar plateau of the closing-volume tracing. Thus, more complex tests may not be essential to studies concerned primarily with verifying exposure effects.

Experimental studies of effects of air pollutants on human health are by nature controversial. Results will be closely examined by proponents of both lower and higher pollution standards, as well as by representatives of various nonmedical disciplines. We report here in detail the experimental approach to a comprehensive investigation of air pollutant health effects and the scientific and practical ration-

ale for this approach. We have, in addition, filed comprehensive data tables with the sponsoring agencies in the form of an "Operational Summary." We hope this detailed documentation can help to dispel doubts concerning the reliability of pollutant exposure studies and promote progress in this field.

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Charles E. Spier, BS, Leonard Wightman, Julie Patterson, MS, Howard Greenberg, BS, and Melvin White gave technical assistance. Dale Hutchinson, PhD (deceased), and other staff members and consultants of the California Air Resources Board aided in this study.

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Experimental Studies on Human Health Effects of Air Pollutants

II. Four-Hour Exposure to Ozone Alone and in Combination With Other Pollutant Gases

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Eight adult male volunteers were exposed to ozone (O_3) alone and then in combination with nitrogen dioxide and carbon monoxide under conditions simulating ambient air pollution exposures.

Four "normal" men showed few or no effects from repeated exposures. Four

male volunteers with a history of "hyper-reactive" airways, but with normal baseline pulmonary function spirometric studies, after O_3 exposure developed definite symptoms and decrement in pulmonary function.

Ozone (O_3) is a major component of photochemical smog. High smog levels occur frequently in the Los Angeles region and are reported in many other urban areas. Other pollutant gases include nitrogen dioxide (NO_2) and organic peroxides.¹ Ozone is one of the most powerful oxidizing agents in smog, and its well-known toxicity in industrial exposures² makes it a pollutant of great concern in air quality protection. Bates and his co-workers³ and Hazucha et al⁴ have reported that normal adults performing intermittent light exercise developed marked pulmonary function decrement from two hours' exposure to O_3 at concentration levels of 0.75 ppm, and measurable decrement from similar exposures at 0.37 ppm. For comparison, the highest one-hour av-

erage oxidant concentration reported in the Los Angeles area between 1963 and 1973⁵ was 0.71 ppm. On the other hand, subjective experience in the Los Angeles area suggests that the majority of citizens are not greatly affected by ambient oxidant concentrations near 0.5 ppm. Furthermore, other experimental studies of exposure^{6,7} have not found marked effects or changes in spirometric measures at concentrations of 0.5 ppm and below. Possible explanations for these apparent discrepancies include differences in exposure time, subject activity, and subject sensitivity, as well as the possible presence of interfering substances in the environment. The present study was undertaken to repeat the previous work under more highly controlled conditions and to expand the scope of investigation to include biochemical tests of blood and tests of psychomotor performance. The experimental plan called for studies of "normal" subjects first, and then testing suspected "hyperreactive" subjects if only minimal effects occurred in normals.

METHODS

The detailed experimental protocol and rationale are given in the preceding article.⁸ Four normal male volunteer subjects (with no prestudy history of cough, chest

discomfort, or wheezing associated with allergy or exposure to air pollution) were recruited from the project investigators or technical staff (designated group 1) and were tested five days per week for three successive weeks. The first three days of each week were devoted to control runs (exposure to purified air); pollutant exposures took place on the fourth and fifth days. Conditions were designed to simulate a composite of extreme ambient conditions experienced during a Los Angeles summer day. Exposures were: first week, 0.50 ppm O_3 ; second week, 0.50 ppm O_3 plus 0.30 ppm NO_2 ; third week, 0.50 ppm O_3 plus 0.30 ppm NO_2 plus 30 ppm carbon monoxide (CO). The variability of the mean concentration of each pollutant over different exposure days was $\pm 5\%$ of the nominal value. During all exposures maximum variation of pollutant concentrations was ± 0.06 ppm for O_3 and NO_2 , and ± 3 ppm for CO. Temperature was held at 31 C and relative humidity at 35%. Each exposure lasted four hours before testing was started and continued during the following hour, or for the period required to complete testing. Although similar ambient conditions occur in the Los Angeles region, the exposure time was approximately twice as long as commonly experienced during severe ambient pollution episodes. This exposure was designed to support or reject the null hypothesis that no effects would be found under the "worst" applied stress. Functions evaluated were forced vital capacity (FVC), one-second forced expiratory volume (FEV₁), partial and maximum forced expiratory flow-volume curves, total respiratory resistance (R_t) by the forced oscillation method, closing volume (CV), alveolar plateau slope from single-breath nitrogen test (delta-nitrogen or ΔN_2), plethysmographic thoracic gas volume (TGV) and airway resistance (R_{aw}), 3-breath rebreathing residual volume (RV),⁹ blood carboxyhemoglobin (COHb) concentration estimate, resting and exercise oxygen consumption ($\dot{V}O_2$), static and dynamic lung compliance (C_{st} , C_{dyn}), and pulmonary diffusing capacity (DL_{CO}). Data were analyzed by repeated measures one-way analysis of variance, comparing the individual's and the group's performance on control days, first exposure days, and second (successive) exposure days. All lung volumes are expressed as BTPS. Smokers were not asked to alter their smoking habits, but no smoking was permitted in the main chamber or during testing.

Because only minimal effects occurred in the normal group, four additional male subjects (designated group 2) were recruited from the project investigators or technical staff and were tested as de-

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scribed above, except pollutant exposures were modified. These subjects had normal FVC, FEV₁, and CV, but had prestudy histories of cough, chest discomfort, or wheezing, associated with allergy or exposure to air pollution. Marked effects developed among some of these subjects during the first exposure to 0.50 ppm O₃. Accordingly, the second 0.50 ppm exposure period (week one, day five) was shortened to two hours for three of the four subjects. In light of the results in week one, the protocol was further modified to look at dose-response relations rather than possible effects of additional pollutants. The subjects were exposed to 0.25 ppm O₃ on two successive days in week two and to 0.37 ppm O₃ on one day in week three.

RESULTS

Biochemical and behavioral findings are reported elsewhere E. E. Pedersen, unpublished data.¹⁰

Few important pulmonary function changes or respiratory symptoms were detected in group 1 under any exposure condition. The physiological importance of the changes in pulmonary function data is in doubt, since the changes occurred in the less stable measurements (R_t, C_{dyn}, DL_{CO}), were small in magnitude, and did not occur consistently throughout all exposures. Statistical analyses for this group are given in Table 1. In group 2, numerous changes were observed with O₃ exposure, as indicated in Table 2 and Fig 1 to 4. At 0.5 ppm, all four subjects developed marked respiratory symptoms and physiological changes, and felt physically ill to the extent that they were unable to perform normal tasks adequately. No such effects were manifested at 0.25 ppm. At 0.37 ppm, physiological changes were relatively mild but symptoms were more severe than at 0.5 ppm.

Individual responses to exposures were variable, but some consistent patterns emerged. These are discussed below along with important individual findings. Prestudy characteristics of individual subjects are given in Table 3.

FVC, FEV₁, and Maximum Expiratory Flow Rates

These functions were consistently reduced in sensitive (group 2) subjects. Contributing factors appeared to be increased airway resistance,

Measure	Exposure	F*	df†	P‡
FVC	O	2.04	2,6	NS
	ON	4.17	2,6	NS
	ONC	<1.0	2,6	NS
V ₉₀	O	1.25	2,6	NS
	ON	<1.0	2,6	NS
	ONC	<1.0	2,6	NS
V ₂₅	O	<1.0	2,6	NS
	ON	<1.0	2,6	NS
	ONC	n/a	n/a	n/a
RV, single breath	O	1.18	2,6	NS
	ON	<1.0	2,6	NS
	ONC	7.73	2,6	<.05§
TLC, single breath	O	6.79	2,6	<.05
	ON	<1.0	2,6	NS
	ONC	<1.0	2,6	NS
DL _{CO}	O	11.1	2,22	<.01¶
	ON	<1.0	2,22	NS
	ONC	15.9	2,22	<.01
Rt, 3 hr	O	<1.0	2,6	NS
	ON	<1.0	2,6	NS
	ONC	7.95	2,6	<.05 , #
CV	O	<1.0	2,22	NS
	ON	3.12	2,22	NS
	ONC	<1.0	2,22	NS
C _{dyn} , normal frequency	O	<1.0	2,78	NS
	ON	<1.0	2,72	NS
	ONC	1.79	2,72	NS
C _{dyn} , 60/min	O	5.62	2,78	<.01¶
	ON	<1.0	2,72	NS
	ONC	2.42	2,72	NS
C _{dyn} , 100/min	O	1.45	2,78	NS
	ON	<1.0	2,78	NS
	ONC	<1.0	2,78	NS
C _{1st}	O	<1.0	2,22	NS
	ON	1.06	2,22	NS
	ONC	<1.0	2,22	NS
TGV (RV)	O	5.67	2,16	<.05 , #
	ON	4.07	2,16	<.05¶, #
	ONC	<1.0	2,16	NS
TGV (TLC)	O	5.47	2,16	<.05 , #
	ON	5.11	2,16	<.05¶, #
	ONC	2.06	2,16	NS

* F = statistic for analysis of variance.

† df = degrees of freedom for analysis of variance.

‡ P = probability of control-exposure difference being due to chance.

§ Decreased first day of exposure.

|| Increased second day of exposure.

¶ Decreased second day of exposure.

Increased first day of exposure.

reduced inspiratory capacity due to substernal pain, and tendency to cough during forced expirations. As these measurements were highly reproducible under control conditions, observed changes with exposure attained higher levels of statistical significance than was true of most other tests.

Lung volumes

Total lung capacity was consistently reduced in sensitive subjects. Residual volume, as determined by three-breath nitrogen dilution,⁹ single-breath nitrogen dilution,¹¹ or plethysmography,¹² increased significantly in subject 7 and was unchanged in the other sensitive subjects. These

Table 2.—Mean Values and Results of Variance Analysis for Physiological Measures: Group 2

Measure	G, Exposure	Control	Exposure 1	Exposure 2	F*	df†	P‡
FVC	.50	5.03	4.55§	4.69§	5.17	2,22	<.05
	.25	5.08	4.94	5.13	<1.0	2,22	NS
	.37	5.07	5.04	None	<1.0	1,11	NS
FEV ₁	.50	3.96	3.51	3.68	§	...	...
	.25	4.07	4.03	4.11	<1.0	2,22	NS
	.37	4.07	3.95	None	2.15	1,10	NS
V ₃₀	.50	4.32	3.42§	3.27*§	4.03	2,14	<.05
	.25	4.09	4.24	4.33	2.01	2,14	NS
	.37	4.15	4.10	None	<1.0	1,8	NS
V ₂₅	.50	1.99	1.50	1.20*§	6.06	2,6	<.05
	.25	1.72	1.67	2.42	2.26	2,10	NS
	.37	1.80	1.78	None	<1.0	1,7	NS
V ₃₀ , partial	.50	4.55	3.14§	3.05*§	5.34	2,16	<.05
	.25	4.66	4.56	4.80	<1.0	2,16	NS
	.37	4.42	3.52	None	8.49	1,9	<.01
V ₂₅ , partial	.50	1.83	1.15	1.19	7.67	2,22	<.01
	.25	2.00	1.71	1.72	1.39	2,20	NS
	.37	1.62	1.39	None	2.22	1,11	<.01
CC	.50	2.35	2.27	2.47	3.06	2,20	NS
	.25	2.33	2.36	2.20	1.05	2,22	NS
	.37	2.21	2.28	None	2.07	1,11	NS
ΔN ₂	.50	0.73	1.60§	1.03	4.21	2,20	<.05
	.25	0.67	0.71	0.63	2.07	2,22	NS
	.37	0.69	0.84	None	2.13	1,11	NS
TLC, single br	.50	6.84	6.42	6.18*§	3.96	2,20	<.05
	.25	6.91	6.85	6.77	<1.0	2,22	NS
	.37	6.85	6.83	None	<1.0	1,11	NS
RV, single br	.50	1.69	1.86	1.76	1.23	2,20	NS
	.25	1.66	1.64	1.58	1.51	2,22	NS
	.37	1.60	1.74§	None	8.17	1,11	<.05
R _{rs} (FRC)	.50	1.26	1.70	1.85	2.50	2,22	NS
	.25	1.09	1.24	1.11	<1.0	2,22	NS
	.37	1.35	1.49	None	2.75	1,11	NS
C _{0.1} , normal frequency	.50	.196	.187	None	<1.0	1,32	NS
	.25	.241	.227	.266	<1.0	2,68	NS
	.37	.224	.222	None	<1.0	1,36	NS
C _{0.1} , 100/min	.50	.179	.153	None	1.68	1,30	NS
	.25	.184	.189	.172	<1.0	2,60	NS
	.37	.173	.127	None	3.07	1,39	NS
DL _{CO}	.50	41.8	42.4	38.8	2.18	2,20	NS
	.25	41.4	40.4	49.8	57.8	2,16	<.01
	.37	40.1	45.4	None	19.4	1,11	<.01

* F = statistic for analysis of variance.

† df = degrees of freedom for analysis of variance.

‡ P = probability of control-exposure difference being due to chance.

§ Significant change from control, P < .05.

|| Significant change from control, P < .01.

¶ Insufficient data, some subjects unable to complete specified number of tests satisfactorily.

changes appear to be due to pain produced by attempting to reach extremes of lung volume.

Single-Breath Nitrogen Test

This test determines phase-4 volume (closing volume), presumably affected by changes in dependent peripheral airways; and phase-3 or

alveolar plateau slope (delta-nitrogen), presumably affected by substantial changes in ventilatory distribution anywhere in the lung. No great changes in closing volume or closing capacity (CV plus RV) were found in any subject in this study, whereas in all sensitive subjects delta-nitrogen values increased with exposure.

Total Pulmonary Resistance

Further investigation is required before results of this test can be fully evaluated, since normal limits and test-to-test variability have not been fully worked out. Substantial changes occurred with 0.5 ppm O₃ exposure, however. Resistance determined by forced oscillation increased at all oscillation frequencies in three of four sensitive subjects and in one "normal" subject (6) in whom no other important physiological changes were found. The increases in oscillatory resistance in the sensitive subjects generally correlated with increases in airway resistance measured plethysmographically. In the fourth sensitive subject (10), total resistance, which was already elevated in comparison with other subjects, did not increase overall but became greatly frequency-dependent, as did dynamic lung compliance (Fig 5), suggesting exposure-related small airways dysfunction. The frequency dependence of resistance persisted through the second and third weeks of the study; this was the only physiological change in any subject that appeared not to return to base line values between the time of exposure and the next following control study.

Lung Compliance

Subject 7 showed a reduction in static compliance with 0.5 ppm exposure, and a corresponding drop in dynamic compliance at all frequencies without substantial frequency-dependence. Subject 10 developed frequency dependence as indicated previously. No other statistically significant changes in compliance were found.

Exercise Testing

No consistent changes in resting or exercise oxygen consumption were found with exposure. One normal subject (6) developed possible angina and minimal electrocardiogram changes during exercise while exposed to CO plus oxidants.

Pulmonary Diffusing Capacity

In both groups 1 and 2, slight decreases in diffusing capacity occurred on the second day of exposure

Table 3.—Subject Characteristics

	Subject No.	Age, yr	Height, cm (Inches)	Weight, kg (lb)	Smoking History Pack-Years*	Prestudy History of Smog Sensitivity†	History of Asthma‡	History of Allergy§	
								Personal	Family
Group 1	1	49	178 (70)	84 (185)	17	0	0	0	0
	3	36	178 (70)	84 (185)	34	0	0	0	0
	4	44	188 (74)	88 (195)	4	0	0	+	0
	6	42	175 (69.5)	84 (185)	50 #	0	0	+	0
	Mean	43	180 (71)	85 (188)	...	...	...	...	...
	(SD)	(5.4)	(5) (2.1)	(2) (5)	...	...	...	...	...
Group 2	7	36	173 (68.5)	70 (155)	0	+	0	+	+
	8	29	172 (68)	71 (157)	0	+	0	0	0
	9	41	188 (74)	80 (177)	20	+	+	+	+
	10	30	172 (68)	78 (172)	30	+	+	+	+
	Mean	34	176 (69.5)	74 (165)	...	...	...	...	...
	(SD)	(5.6)	7 (2.9)	5 (11)	...	...	...	...	...

* One pack year = one pack of cigarettes per day for one year.

† Defined by symptoms of cough, wheezing, or chest discomfort when outside on high pollution days (Los Angeles area).

‡ Defined by spontaneous attacks of bronchospasm requiring bronchodilator therapy separated by asymptomatic intervals.

§ Defined by symptoms of wheezing, gastroenteritis, or dermatitis when in contact with specific antigens or a history of chronic rhinitis, postnasal drip, or hayfever.

|| Now smokes pipe.

|| Still smoking: all others have been nonsmokers for at least four years prior to study.

Also smokes and inhales cigars.

as compared with the first day of exposure, when results were essentially unchanged from control values. Test results appeared less reproducible in this study than expected under normal conditions (resting subjects, normal room temperature).

Symptomatology

Symptoms were absent in group 1 during control runs, but some subjects reported mild pharyngitis or substernal discomfort during exposures. A low frequency of symptoms in group 2 during control runs increased dramatically with O₃ exposure at 0.5 and 0.37 ppm. All four subjects experienced cough, substernal pain, wheezing, and malaise. Cough was generally nonproductive, but subject 7 produced a small amount of blood-streaked sputum during the second exposure at 0.5 ppm. Of the sensitive subjects, only subject 9 was able to complete two successive four-hour exposures; his symptoms and physiological changes were only slightly increased on the second exposure day. The sensitive subjects' symptoms generally moderated soon after exposure was completed but some persisted throughout the remainder of the exposure day, and sometimes throughout the next day.

The project physician assigned symptom scores for each subject on

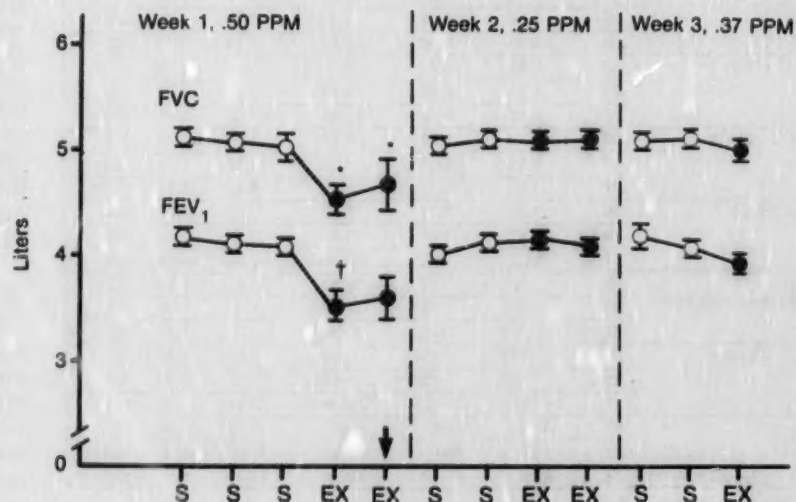


Fig 1.—Daily group means (± 1 SE) for FVC and FEV₁, group 2. Open circles, control, and black circles, O₃ exposure. *Significant change from control, $P < .05$; †Significant change from control, $P < .01$. Arrow, exposure time decreased from four to two hours in three of the four subjects. S = sham exposure, and EX, O₃ exposure.

each day following an interview using a standard questionnaire. Symptoms of cough, wheezing, sputum production, substernal pain, dyspnea, fatigue, headache, laryngitis, and nasal discharge were scored. Each was rated from 0 to 4 points, based on severity, during each of the three time periods—during exposure, remainder of the exposure day, and the following morning. Less specific symptoms such as malaise and muscular aches were

not scored. In group 2, symptom scores were strongly correlated with observed physiological changes except in the 0.37 ppm O₃ exposure, in which symptoms were more severe than would be expected, and out of proportion to the physiological findings. The 0.37 ppm exposure was during the third week and the severity of symptoms at that time was greater than for the 0.5 ppm exposure of the first week.

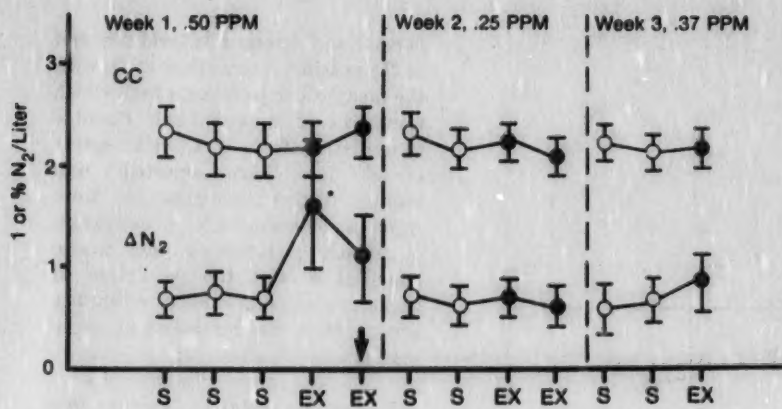


Fig 2.—Daily group means (± 1 SE) for closing capacity (CC) and delta-nitrogen (ΔN_2), group 2. Open circles, control, and black circles, O_2 exposure. *Significant change from control, $P < .05$. Arrow, exposure time decreased from four to two hours in three of the four subjects. S = sham exposure, and EX, O_2 exposure.

COMMENT

A relatively broad range of sensitivity to O_2 has been demonstrated, with observed effects of exposure correlating well with subjects' own opinions concerning their sensitivity. Although the number of subjects tested is small, the results support the hypothesis that individuals with pre-existing pulmonary hyperreactivity are more severely affected by exposure and thus more at risk in polluted environments. All four subjects with any history of pulmonary hyperreactivity were significantly affected by 0.37 ppm O_2 , while all four subjects without such a history were affected minimally or not at all by 0.50 ppm O_2 . In the latter subjects, addition of a second oxidant pollutant, NO_2 , at 0.3 ppm, did not produce additional detectable effects. This does not, however, preclude the possibility that additive or synergistic effects of exposure to O_2 and NO_2 in combination may occur at higher NO_2 concentrations, at higher relative humidity, or in more sensitive subjects. Addition of CO to the pollutant mixture also failed to produce detectable effects other than increases in blood carboxyhemoglobin levels.

A comparison of the present results with the findings of Bates et al¹ and Huzucha et al¹ is in order. As shown in Table 4, the symptoms experienced and most of the changes in pulmonary function observed were similar in the two studies. Discrepancies may be related to differences in methodology rather than in physiological responses. One might infer that Bates' Canadian subjects, although they had no history

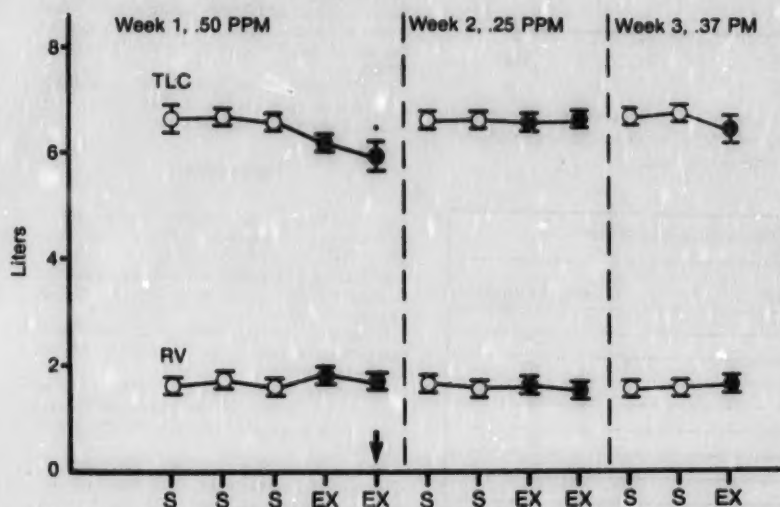


Fig 3.—Daily group means (± 1 SE) for lung volumes calculated from single-breath nitrogen tracings. Open circles, control, and black circles, O_2 exposure. *Significant change from control, $P < .05$. Arrow, exposure time decreased from four to two hours in three of the four subjects. S = sham exposure, and EX, O_2 exposure.

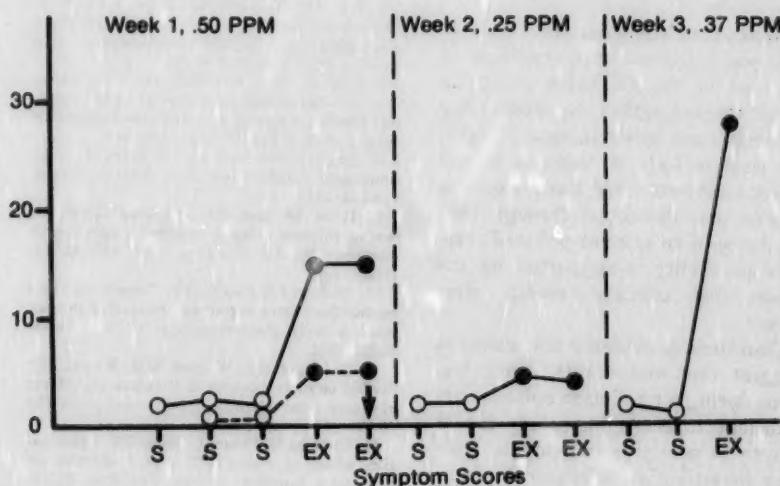


Fig 4.—Daily mean symptom scores related to O_2 exposure for group 1 (dotted line, week 1 only) and group 2 (solid lines). Open circles, control, and black circles, O_2 exposure. Arrow, exposure time decreased from four to two hours in three of four subjects of group 2. S = sham exposure, and EX, O_2 exposure.

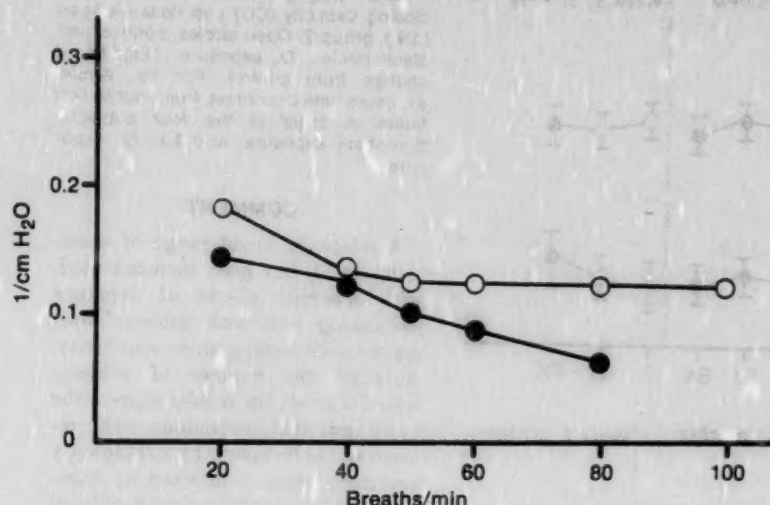


Fig 5.—Dynamic compliance vs breathing frequency, subject 10. Open circles, mean of control runs for week 1, and black circles, first day of 0.50 ppm O₃ exposure. Subject could not perform maneuver at 100 breaths/min under exposure.

Table 4.—Comparison of Typical Effects of O₃ Exposure

Measure	Bates et al ³ & Hazucha et al ⁴	Present Study
FVC	—*	—*
FEV ₁	—*	—*
V ₅₀	—*	—*
CV or CC	++	0‡
TLC	0*	—*
RV	++	0‡
Cough	++	++
Substernal pain	++	++
Wheezing	++	++
Laryngitis	++	++

* — = Decreased.

† + = Increased (or symptoms observed).

‡ 0 = Unchanged in most or all cases.

of respiratory disease, were more sensitive on the average, since they experienced functional decrement similar to the present (Los Angeles) group 2 subjects. The present group 1 subjects, also with no history of respiratory disease, experienced virtually no decrease in function. Several possible explanations for this apparent discrepancy may be suggested. Random variation in sensitivity among individuals may be a factor, given the small numbers of subjects tested. Reportedly similar concentrations of O₃ might actually have been different in the two studies, since different monitoring techniques were used. Additional unknown pollutants modifying the effect of O₃ might have been

present in one study but not the other. This possibility seems more likely in the case of the Canadian study, in which air purification and monitoring apparatus was less elaborate. Finally, the relative lack of response in the present subjects could have been due to tolerance developed through long residence in an oxidant-polluted area. This possibility is supported by the report that animals develop tolerance.¹²

Considerable evidence now exists to suggest that ambient O₃ concentrations during air pollution episodes are sufficient to cause important health effects in sensitive individuals. Further investigation is required of the effects of controlled O₃ exposures in

normal and diseased individuals and of the possible interactions of O₃ with the many other pollutants with which it may coexist in ambient air. Possible synergistic effects of O₃ with sulfur dioxide have been reported¹⁴ and require further investigation. More rigorous epidemiologic investigation of ambient pollution episodes is also essential to test the prediction of important exposure-related morbidity arising from the controlled-exposure studies.

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C. E. Spier, L. Wightman, H. Greenberg, J. Patterson, and Dr. D. C. Law aided with technical assistance and consultation.

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Experimental Studies on Human Health Effects of Air Pollutants

III. Two-Hour Exposure to Ozone Alone and in Combination With Other Pollutant Gases

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Adult male volunteers were exposed to ozone (O_3) at 0.25, 0.37, or 0.50 ppm, and to O_3 in combination with nitrogen dioxide (NO_2) and carbon monoxide (CO), with secondary stresses of heat, intermittent light exercise, and repeated exposure.

Few important physiological changes, and only mild symptoms, were found with 0.25 ppm O_3 , with 0.25 ppm O_3 plus 0.30 ppm NO_2 , or when 30 ppm CO was added to the latter mixture. With 0.37 ppm O_3 ,

more symptoms were present and some subjects developed definite decreases in pulmonary function. With 0.50 ppm O_3 , most subjects had symptoms and about half showed substantial pulmonary function decrement.

In reactive subjects exposed on two successive days, changes were usually greater the second day, indicating that effects of successive exposures were cumulative.

Ozone (O_3) is a common photochemical oxidant air pollutant that may be an important health hazard. Bates and co-workers¹ and Hazucha et al.² found that volunteers having no history of respiratory disease and living in an area with little oxidant

pollution developed substantial respiratory symptoms and function decrement when exposed for two hours to O_3 at concentrations as low as 0.37 ppm, with intermittent light exercise. In previous work in this laboratory,^{3,4} similar studies were conducted on volunteers living in the Los Angeles area, where ambient O_3 concentrations of 0.5 ppm or higher are possible. Four "normal" subjects (without a prestudy history of cough, chest discomfort, or wheezing associated with allergy or air pollution exposure) failed to react to O_3 exposure at 0.50

ppm, even when exposed for four-hour periods on two successive days. Four "reactive" subjects (with a prestudy history of cough, chest discomfort, or wheezing associated with allergy or air pollution exposure, but with normal base line pulmonary function studies) during one four-hour exposure to 0.50 ppm O_3 developed substantial decrease in pulmonary function and symptoms severe enough to restrict normal activity. The same subjects did not react appreciably to 0.25 ppm O_3 , but did react somewhat to 0.37 ppm O_3 . The degree of pulmonary function decrement found in reactive subjects led us to decrease the exposure time to two hours. The present studies use a two-hour exposure time and were undertaken to better define the range of sensitivity and dose-response characteristics in "normal" and "reactive" populations. Such information is required as a basis for setting realistic air quality standards for the protection of public health.

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Table 1.—Subject Characteristics

Subject No.	Exposure Group(s)	Age, yr	Height, cm (in)	Weight, kg (lb)	Smoking History Pack-Years*	Prestudy History of Smog Sensitivity†	History of Asthma‡	History of Allergy§	
								Personal	Family
3	3	36	178 (70)	84 (185)	34	0	0	0	0
7	4	36	173 (68.5)	70 (155)	0	+	0	+	+
8	5	29	173 (68)	71 (157)	0	+	0	0	0
9	4,5	41	188 (74)	80 (177)	20	+	+	+	+
10	4,5	30	173 (68)	78 (172)	30	+	+	+	+
11	3,4	30	183 (72)	70 (155)	0	0#	0	0	0
12	3	28	183 (72)	75 (166)	0	0	0	0	+
13	3	28	188 (74.5)	70 (155)	0	0	0	+	+
14	3	34	175 (69.5)	68 (150)	0	0	0	0	0
15	3,4	22	165 (65)	58 (128)	1	0#	0	+	+
16	3,4	30	183 (72)	79 (175)	7.5	0#	0	0	0
17	4,5	41	167 (66)	64 (142)	0	0	0	0	0
18	5	27	178 (70)	68 (150)	0	0	0	0	0

* One pack year = one pack of cigarettes per day for one year.

† Defined by symptoms of cough, wheezing, or chest discomfort when outside on high pollution days (Los Angeles area).

‡ Defined by spontaneous attacks of bronchospasm requiring bronchodilator therapy separated by asymptomatic intervals.

§ Defined by symptoms of wheezing, gastroenteritis, or dermatitis when in contact with specific antigens or a history of chronic rhinitis, postnasal drip, or hay fever.

|| Now smokes pipe.

Still smoking; all others have been nonsmokers for at least four years prior to study.

Following the chamber study a close reevaluation of their history suggested that these subjects should be classified as smog sensitive.

Table 2.—Mean Values and Results of Variance Analysis for Physiological Measurements, Group 3 (0.50 ppm O₃ Exposure)

Measure	Control	Exposure 1	Exposure 2	F	df*	P
FVC	5.15	5.03	4.75†	6.46	2,38	<.01
FEV ₁	4.46	4.25	3.86†	7.56	2,38	<.01
V _{max}	12.0	11.7	10.9‡	3.52	2,38	<.05
V ₂₅	5.95	5.73	4.84†	7.33	2,34	<.01
V ₂₅	2.69	2.39‡	2.20†	6.86	2,34	<.01
V ₂₅ P	6.42	6.08	5.12†	13.5	2,34	<.01
V ₂₅ P	2.68	2.54	2.21†	6.68	2,38	<.01
CC	1.85	1.81	1.86	<1.0	2,38	NS§
ΔN ₂	0.66	0.75	1.04‡	4.64	2,38	<.05
TLC, single-breath	6.64	6.44	5.97†	5.29	2,38	<.01
RV, single-breath	1.41	1.32	1.38	2.42	2,38	NS§
RV, rebreathing	1.66	1.66	1.70	<1.0	2,40	NS§
R _L , 6 hz	3.39	3.52	4.77†	5.39	2,98	<.01
DL _{CO}	39.2	38.8	Not available			

* df = degrees of freedom.

† Significant change from control, $P < .01$.

‡ Significant change from control, $P < .05$.

§ NS = change not significant at .05 level.

METHODS

The controlled-environment exposure facility and detailed experimental protocol used for these studies have been described previously.¹ Thirteen volunteer subjects, recruited from the project professional and technical staff, participated in one or more exposure groups (Table 1). Group 3, consisting of seven "normal" subjects (without a prestudy history of cough, chest discomfort, or wheezing associated with allergy or air pollution exposure), was exposed to 0.50 ppm O₃ in a one-week study. (Groups 1 and

2 are described in the previous report¹). Groups 4 and 5 included both "normal" and "reactive" subjects. In a three-week study, group 4 was exposed to 0.25 ppm O₃ the first week, to 0.25 ppm O₃ plus 0.30 ppm NO₂ the second week, and to 0.25 ppm O₃ plus 0.30 ppm NO₂ plus 30 ppm CO the third week. Group 5 was exposed to 0.37 ppm O₃ in a one-week study. During each week of studies, the first two or three days were devoted to sham exposures (exposures to purified air) to establish base line measures of functions, and the final two days were devoted to pollutant exposures (the

given concentration of pollutant added to purified air).

Each subject was exposed in the controlled-environment chamber for two hours, and then performed the battery of physiological tests while still under exposure. During the two-hour period, the first 15 minutes of every 30 was spent in exercise at a level sufficient approximately to double the volume of resting ventilation per minute. Exercise consisted of walking briskly or riding a bicycle ergometer. Each subject also recorded symptoms on a standard form. After completing the physiological tests, the subject left the exposure chamber and was immediately examined by the project physician. A venous blood sample was taken for biochemical analysis and the subject was interviewed for symptoms according to a standard questionnaire. Symptoms experienced during the remainder of the day were also recorded on a standard form.

Physiological results evaluated were forced vital capacity (FVC); one-second forced expiratory volume (FEV₁); partial and maximum forced expiratory flow-volume curves; total respiratory resistance by forced oscillation at 3, 6, 9, and 12 hertz (R_L); closing volume (CV); lung volume estimates, and slope of the alveolar plateau (ΔN₂/liter) from the single-breath nitrogen test; carboxyhemoglobin (COHb) concentration estimate by breath analysis; and single-breath carbon monoxide diffusing capacity (DL_{CO}). Supplementary physiological tests included plethysmographic thoracic gas volume (TGV) and airway resist-

Fig 1.—Daily variation of pulmonary functions in group 3, mean $\pm$ 1 SE. S, sham exposure, and EX = 0.5 ppm O_3 exposure. TLC, RV, CC, and ΔN_2 calculated from single-breath nitrogen tracings.

ance (R_{rs}) (groups 4 and 5), static (C_{st}) and dynamic (C_{dyn}) lung compliance (group 5), and residual volume (RV) by rebreathing (group 3). Psychomotor performance was evaluated in group 4 through measurement of reaction time, heart rate variability, and tracking performance, in a combined central and peripheral tracking task (E. E. Pedersen et al, unpublished data). Data for each exposure group were analyzed using a repeated measures, one-way analysis of variance as described previously.²

RESULTS Group 3

This "normal" group showed decreases, after 0.5 ppm O_3 exposure, in forced expiratory measurements including FVC, FEV₁, flow rate at 50% FVC on maximum and partial forced expiratory flow-volume curves ($\dot{V}_{50}$ and $\dot{V}_{50}$ P, respectively), and flow rates on flow-volume curves at 25% FVC ($\dot{V}_{25}$ and $\dot{V}_{25}$ P). Closing volume and closing capacity as measured by the single-breath nitrogen test did not change, but the alveolar nitrogen plateau slope (ΔN_2) increased, indicating less uniform distribution of ventilation as a result of exposure. Residual volume measurement by rebreathing nitrogen dilution³ produced larger values than were obtained from the single-breath nitrogen RV estimate, but neither measurement indicated significant changes in RV with exposure. Total lung capacity (TLC) decreased due to the decrease in vital capacity. Exposure increased total pulmonary resistance at all oscillation frequencies.

The group changes in physiological measurements, in many cases, did not achieve statistical significance until the second exposure day, and in most cases the decrements in function were markedly worse on the second day. The group changes were primarily due to subjects 11, 13, and 16. The remaining four subjects (including one

Fig 3.—Daily variation of lung volumes, calculated from single-breath nitrogen tracings, in group 4, mean $\pm$ 1 SE error (see Fig 2 for explanation).

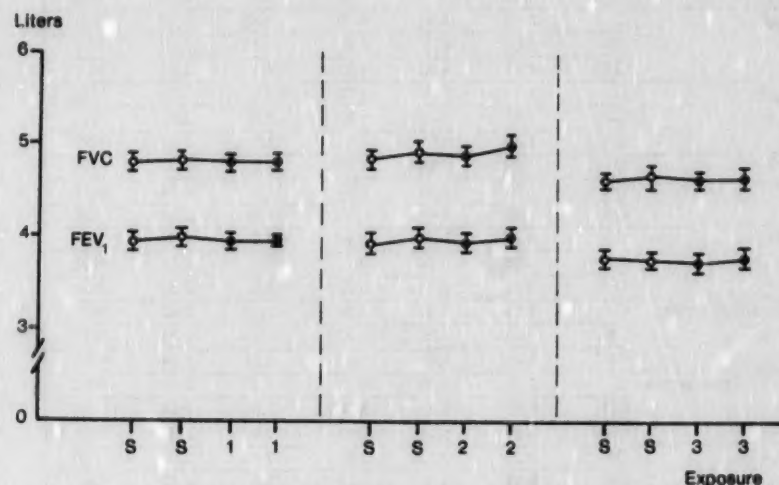
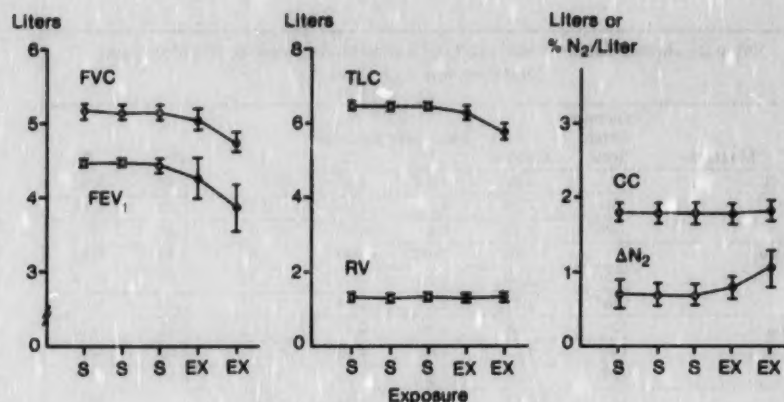


Fig 2.—Daily variation of forced expiratory measurements in group 4, mean $\pm$ 1 SE. Exposure conditions: S = sham; 1 = 0.25 ppm O_3 ; 2 = 0.25 ppm O_3 + 0.30 ppm NO_2 ; 3 = 0.25 ppm O_3 + 0.30 ppm NO_2 + 30 ppm CO. Successive weeks of study are separated by broken lines. Subject 9 absent week three; subject 11 absent week two.

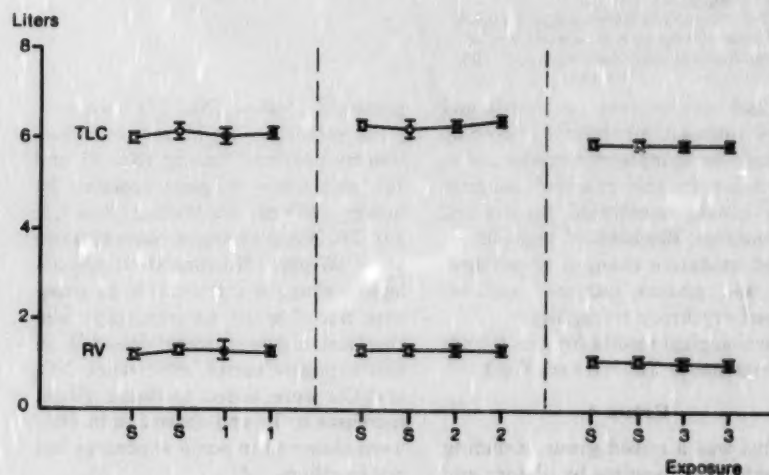


Table 3.—Mean Values and Results of Variance Analysis for Physiological Measurements, Group 4

Measure	Exposure Conditions*	Control	Exposure 1	Exposure 2	F	df†	P
FVC	O	4.82	4.80	4.79	<1.0	2,40	NS‡
	ON	4.88	4.88	5.01§	6.85	2,32	<.01
	ONC	4.86	4.84	4.68	<1.0	2,32	NS‡
FEV ₁	O	3.94	3.95	3.96	<1.0	2,38	NS‡
	ON	3.94	3.96	4.03	4.09	2,30	<.05
	ONC	3.77	3.74	3.79	1.67	2,32	NS‡
V ₅₀	O	4.66	4.66	4.84	1.50	2,12	NS‡
	ON	4.63	4.47	4.48	<1.0	2,10	NS‡
	ONC	4.58	4.58	4.63	<1.0	2,10	NS‡
V ₂₅	O	2.01	2.07	2.10	<1.0	2,12	NS‡
	ON	1.91	1.85	1.87	<1.0	2,10	NS‡
	ONC	1.86	1.77	1.82	<1.0	2,10	NS‡
V ₅₀ P	O	5.12	4.91	5.10	1.45	2,12	NS‡
	ON	4.67	4.62	4.92	2.55	2,10	NS‡
	ONC	4.91	4.93	4.83	<1.0	2,10	NS‡
V ₂₅ P	O	2.11	2.07	2.10	<1.0	2,12	NS‡
	ON	1.91	1.85	1.93	<1.0	2,10	NS‡
	ONC	1.87	1.78	1.92	<1.0	2,10	NS‡
CC	O	1.88	1.85	1.77§	10.7	2,40	<.01
	ON	1.98	1.93	1.93	<1.0	2,34	NS‡
	ONC	1.63	1.64	1.65	<1.0	2,34	NS‡
ΔN ₂	O	0.70	0.68	0.66	<1.0	2,40	NS‡
	ON	0.75	0.74	0.72	<1.0	2,34	NS‡
	ONC	0.72	0.75	0.72	<1.0	2,32	NS‡
RV, single-breath	O	1.30	1.37	1.32	2.13	2,40	NS‡
	ON	1.40	1.38	1.36	1.74	2,34	NS‡
	ONC	1.15	1.11	1.11	2.01	2,34	NS‡
TLC, single-breath	O	6.27	6.25	6.33	1.34	2,40	NS‡
	ON	6.50	6.53	6.66§	7.91	2,34	<.01
	ONC	6.16	6.14	6.12	<1.0	2,34	NS‡
R _s , 6 hz	O	3.25	3.83§	3.07	7.11	2,34	<.01
	ON	3.33	3.50	3.28	<1.0	2,32	NS‡
	ONC	3.35	3.65	3.54	<1.0	2,30	NS‡
DL _{CO}	O	40.4	42.0	34.2	4.61	2,40	<.05
	ON	45.0	36.6§	39.5	6.27	2,34	<.01
	ONC	36.2	38.4	38.0	<1.0	2,32	NS‡

* Exposure conditions: O = 0.25 ppm O₃; N = 0.3 ppm NO₂; C = 30 ppm CO.

† df = degrees of freedom.

‡ NS = change not significant at .05 level.

§ Significant change from control, *P* < .01.

|| Significant change from control, *P* < .05.

who had been exposed previously and found nonreactive) showed few or no changes or symptoms. Predominant symptoms in the reactive subjects were cough, substernal discomfort, and malaise. Biochemical analysis revealed oxidative changes in erythrocyte and plasma enzymes and increased erythrocyte fragility.*

Physiological results for group 3 are summarized in Table 2 and Fig 1.

Group 4

This was a mixed group including one subject unreactive by history and

previously tested (No. 17), two subjects unreactive by history but reactive by previous testing (No. 11 and 16), and three subjects reactive by history and previous testing (Nos. 7, 9, and 10). Ozone exposure concentration was 0.25 ppm. No consistent physiological changes attributable to exposure and few or no important biochemical changes were detected in this exposure series, even when NO₂ and CO were added to ozone. Slight increases in R_s and decreases in DL_{CO} were observed in some exposures but not in others.

Physiological results for group 4 are summarized in Table 3 and in Fig 2 to 4.

Group 5

This group included three subjects reactive by history and previous testing (No. 8, 9, and 10) and two subjects (No. 17 and 18) unreactive by history but not tested previously at or above the O₃ concentration of 0.37 ppm. In this group important changes with exposure in most physiological measures were not found. However, subject 8 showed substantial decreases in FVC and FEV₁ and increases in R_s and airway resistance (R_{aw}) at functional residual capacity (FRC) measured plethysmographically. These changes became worse on the second exposure day. For the group, R_{aw} at FRC did not change substantially with exposure, but R_s was increased above control on both exposure days. Symptoms reported by subject 8 were similar to those of the reactive subjects in group 3. The remaining subjects most frequently reported upper-airway irritation and coughing. Oxidative biochemical changes were detected in blood of this group, but were not as severe as in groups exposed to 0.5 ppm O₃.

Physiological results for group 5 are summarized in Table 4 and Fig 5.

COMMENT

The foregoing results, together with those reported previously from this laboratory,^{1,2} allow formulation of dose-response curves for effects of O₃ exposure (Fig 6 to 8) based on less variable physiological and biochemical measurements. Equation of these observed responses with "important deleterious effects on health" is not completely straightforward, since the underlying mechanisms are not fully understood. However, from a practical standpoint the observed physiological changes may reasonably be considered to represent important health effects, since (a) the changes are qualitatively similar to those observed in certain pulmonary diseases and (b) in studies to date, substantial physiological changes have always been accompanied by marked clinical illness (respiratory symptoms severe enough to

Fig 4.—Daily variation of closing capacity and ΔN_2 in group 4, mean $\pm$ 1 SE (see Fig 2 for explanation).

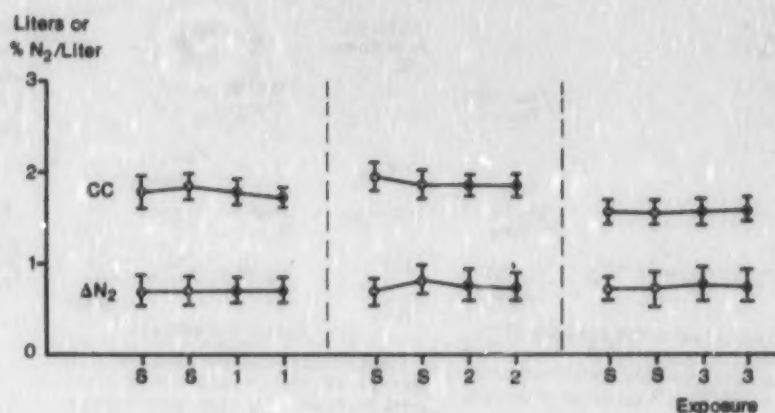


Fig 5.—Daily variation of pulmonary functions in group 5, mean $\pm$ 1 SE. S = sham exposure, and EX = 0.37 ppm O_3 exposure. TLC, RV, CC, and ΔN_2 calculated from single-breath nitrogen tracings.

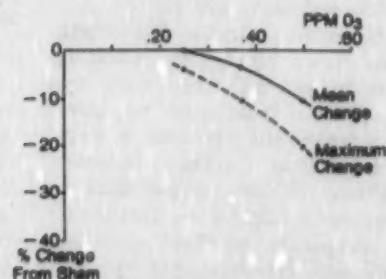
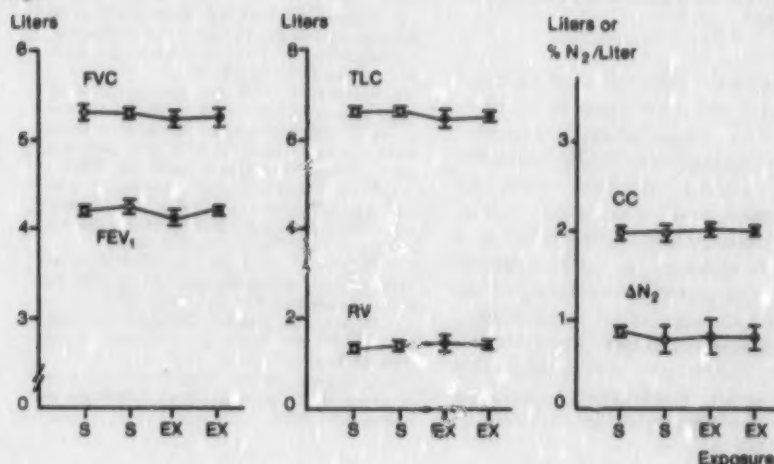


Fig 6.—Dose-response behavior of FEV_1 in subjects exposed to O_3 . Mean and maximum changes from control values observed in all subjects tested at given concentrations.

inhibit normal activity). While biochemical changes have been observed in asymptomatic as well as symptomatic subjects, the dose-response curves for those biochemical measurements expected to result in an immediate response to oxidant exposure are remarkably similar to the curves for the physiological measurements.

The given dose-response curves are to be considered first approximations only, since they are based on small samples, neglect differences in exposure time, and do not distinguish between initial and cumulative exposures. Responses are expressed in units of percent change from the appropriate control values. The mean of individual responses and the large-

Table 4.—Mean Values and Results of Variance Analysis for Physiological Measurements, Group 5 (0.37 ppm O_3 Exposure)

Measure	Control	Exposure 1	Exposure 2	F	df*	P
FVC	5.37	5.29	5.35	<1.0	2,8	NS†
FEV_1	4.29	4.19	4.24	1.11	2,8	NS†
$\dot{V}_E$	4.94	4.74	4.68	<1.0	2,8	NS†
$\dot{V}_E/\dot{V}_T$	2.15	1.9	1.9	(Not available)		
$\dot{V}_E/P$	5.49	4.66	4.50	3.03	2,8	NS†
$\dot{V}_T/P$	2.18	1.86	1.86	10.3	2,8	NS†
CC	1.97	2.04	2.02	<1.0	2,8	NS†
ΔN_2	0.80	0.79	0.81	<1.0	2,28	NS†
TLC, single-breath	6.81	6.96	6.75	<1.0	2,26	NS†
R_{rs} , FRC	1.40	1.55	1.48	<1.0	2,28	NS†
R_{rs} , 6 hz	3.24	4.01‡	3.88‡	7.25	2,26	<.01
$C_{d_{20}}$, 20/min	0.282	0.288	0.290	<1.0	2,80	NS†
$C_{d_{100}}$, 100/min	0.136	0.130	0.129	<1.0	2,97	NS†

* df = degrees of freedom.

† NS = not significant at .05 level.

‡ Significant change from control, $P < .01$.

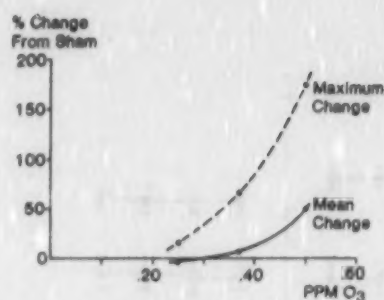


Fig 7.—Dose-response behavior of ΔN_2 in subjects exposed to O_3 . Mean and maximum changes from control values observed in all subjects tested at given concentrations.

est individual response observed at each concentration are plotted as a function of concentration and smooth curves drawn through the resulting points to generate the "mean response" and "maximum response" curves for the study population. Best-fit straight lines have also been generated from the dose-response data by least-squares calculations; these indicate comparable no-effect levels but appear to overestimate the response at the intermediate concentration. The mean dose-response curves suggest a "no-detectable-effect" level of 0.25 to 0.30 ppm. Ambient oxidant concentrations exceed 0.30 ppm for one hour or more at least 20 days per

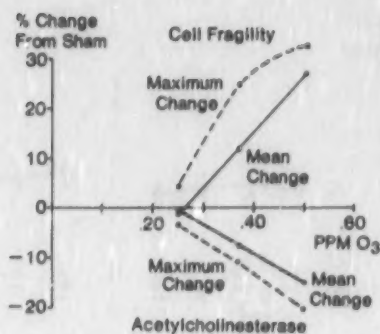


Fig 8.—Dose-response behavior of selected erythrocyte measurements in subjects exposed to O_3 . Mean and maximum changes from control values observed in all subjects tested at given concentrations. Black circles, fragility of cells, and open circles, cell membrane acetylcholinesterase level.

year in parts of the Los Angeles area,* and such levels are possible in many other urban areas. Since the exposure conditions that were studied simulate light, outdoor, physical work, an important and widespread public-health risk related to O_3 pollution is implied, although the precise comparability of exposures to O_3 in purified air and to complex ambient oxidant mixtures is not known. Furthermore, many individuals are considerably more sensitive than the average, and thus may be at risk at considerably

lower levels of pollution. The degree of risk to types of populations not studied, such as children, the elderly, or chronic pulmonary disease patients remains to be determined.

This study was supported by a contract from the California Air Resources Board, No. 2-372, a National Heart and Lung Institute Specialized Center of Research grant, HL ES 15096, and an Environmental Protection Agency grant, R-801396.

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HEALTH EFFECTS OF OZONE EXPOSURE IN CANADIANS VS. SOUTHERN CALIFORNIANS:
EVIDENCE FOR ADAPTATION?

RUNNING TITLE: HEALTH EFFECTS OF OZONE

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ABSTRACT

Comparison of published reports on health effects of exposure to ozone (O_3) suggests that Canadians are more reactive than Southern Californians. Subject responses and experimental methods were compared further in a cooperative investigation of this apparent reactivity difference. Four Canadians and four Californians were exposed to 0.37 ppm O_3 in purified air at 21°C and 50 percent relative humidity for two hours with intermittent light exercise. Exposures to purified air alone served as controls. Subject responses were similar to those observed previously--Canadians on the average showed greater clinical and physiological reactivity to exposure than did Californians, who were no more than minimally reactive. Canadians also showed larger increases in erythrocyte fragility following exposure. Methodological differences sufficient to explain different results of previous studies were not found. Although other possible explanations have not been entirely ruled out, adaptation of Southern Californians to chronic ambient O_3 exposure is a rational hypothesis which can explain these results.

HEALTH EFFECTS OF OZONE EXPOSURE IN CANADIANS VS. SOUTHERN CALIFORNIANS:
EVIDENCE FOR ADAPTATION?

Introduction

Previous work in our respective separate laboratories¹⁻⁷ has shown that significant adverse physiological, clinical, and biochemical changes occur in humans exposed to ozone (O_3) under conditions realistically simulating ambient exposures experienced during photochemical smog episodes. Dose-response information obtained from these studies indicates that typical Montreal or Toronto residents show at least twice as great a response to a given O_3 dose as do typical Los Angeles residents studied under presumably similar conditions, when response is expressed as decrement in a pulmonary function measurement such as one-second forced expiratory volume (FEV_1). It was hypothesized that differences in reactivity might be due to development of biological adaptation in Los Angeles subjects, somewhat analogous to tolerance observed in experimental animals.⁸ (We use the term "adaptation" in relation to human studies at near-ambient O_3 concentrations, since "tolerance" is generally used in reference to higher concentration animal mortality studies. "Adaptation" in this sense implies a beneficial effect only in the short term, since its long-term effects are unknown.) Metropolitan Los Angeles experiences more frequent O_3 pollution episodes and considerably higher O_3 concentrations than Canadian cities, although the latter are not entirely free of O_3 pollution (Table 1).

Other possible explanations for the different results of Los Angeles and Canadian studies include modification of effects of O_3 by coexisting unknown pollutants, random variation in reactivity among individuals, selective migration of hyperreactive individuals, nutritional differences (known to be relevant in animals⁹), genetic differences,

and differences in O_3 monitoring leading to higher actual doses in the Canadian studies relative to the Los Angeles studies. Significant differences among "standard" O_3 monitoring techniques have recently been documented,¹⁰ pointing up the importance of investigating the last possibility. The hypothesis of adaptation was tested by conducting a cooperative study in which human subjects, monitoring techniques, and physiological measurement techniques used in the different laboratories were directly compared. Goals were (a) to confirm the difference in reactivity between Canadians and Southern Californians by replicating previous results from the separate laboratories, and (b) to support the adaptation hypothesis by ruling out as completely as possible any alternative explanations for the difference in reactivity.

Methods

Subjects were exposed to purified air (control) and to 0.37 parts per million (ppm) O_3 . Exposures lasted two hours, during which subjects exercised for 15 minutes in every 30 at a level sufficient to increase minute ventilation to 2-2 1/2 times the resting level. Exposure temperature was 21°C and relative humidity was 50% ± 4%. Forced expiratory measurements were made at 30-minute intervals during exposure; other physiological tests were performed at the end of the 2-hour period, the exposure continuing during testing. After testing was complete, exposure was stopped. Clinical examinations of the subjects were immediately performed and venous blood drawn for biochemical studies. Details of the physiological and biochemical testing have been described previously.^{1,11}

Of the exposure facilities available, the Los Angeles facility (Rancho Los Amigos Hospital Clinical Environmental Stress Testing Laboratory) had the most elaborate environmental control and monitoring capability,¹¹ thus it was used for the cooperative exposure study. Four volunteer subjects, residents of Toronto and Hamilton, Ontario, traveled to Los Angeles for the study. During their stay the subjects remained in the coastal areas of the Los Angeles Basin to minimize their ambient O_3 exposures. A representative Canadian O_3 monitoring instrument (Mast coulometric analyzer from McMaster University) was also brought to Los Angeles and used to monitor the exposure atmosphere in parallel with the local instruments (REM chemiluminescent analyzer and Beckman continuous colorimetric analyzer). All instruments were originally calibrated to read equivalent to manual samples determined using one percent neutral phosphate-buffered potassium iodide solution.¹² Four Los Angeles subjects, matched as closely as practical to the Canadian subjects, were exposed separately under comparable conditions. Both groups of subjects included smokers, nonsmokers, individuals previously found to be reactive to O_3 exposure, and individuals not previously studied but with histories of respiratory hypersensitivity, which is associated with hyperreactivity to O_3 .⁴ Subject characteristics are summarized in Table 2.

Data were analyzed by t tests or analyses of variance comparing control and post-exposure measurements. In the physiological tests, repeated measures on each individual were possible, thus each individual

as well as each group could be tested for significant changes. In the biochemical analyses, only one value was available for each individual for each condition, thus only group statistical comparisons were made.

Results

Ozone Monitoring. During the exposure of the Canadian subjects, the mean readings of the O₃ monitors were 0.37 ppm for the Los Angeles chemiluminescent instrument, 0.34 ppm for the Los Angeles colorimetric instrument, and 0.30 ppm for the Canadian electrochemical instrument. In a separate experiment with no subjects in the exposure chamber, the three instruments read respectively 0.52, 0.47, and 0.44 ppm. Standard deviations of data recorded at fixed intervals from all instruments did not exceed 0.02 ppm. Relative deviation of the Canadian instrument from the Los Angeles instruments thus ranged from -6 percent to -19 percent. If these data are typical of differences in monitoring between the Canadian and Los Angeles laboratories, monitoring differences can explain some, but certainly not all, of the difference in reactivity between the locations.

Clinical Response. Canadian subjects 19 and 20 developed definite clinical illness during O₃ exposure. Both experienced nonproductive cough, substernal discomfort, and upper-airway irritation (laryngitis or pharyngitis). The symptoms moderated after exposure was terminated, but did not disappear for several hours. These responses were similar to those exhibited by these individuals when previously studied in Canada.^{3,6} Canadian subjects 21 and 22 developed upper-airway irritation during exposure, and subject 22 also reported slight substernal discomfort. Los Angeles subjects 7 and 23 reported upper-airway irritation during exposure; no other exposure-related symptoms were reported by the Los

Angeles subjects. The relative lack of response was consistent with previous findings in Los Angeles subjects exposed at similar concentrations.⁵

Physiological Response. Group mean physiological data are given in Table 3. Neither the Canadian nor the Los Angeles group showed significant differences between control and exposure studies. The clinically reactive Canadian subjects 19 and 20 showed significant ($P<.05$) losses in FEV_1 , forced vital capacity (FVC), and total lung capacity (TLC) as estimated from the single-breath nitrogen washout.¹³ Subject 19 also exhibited a significant increase in delta nitrogen, representing a decrease in uniformity of ventilation distribution. FEV_1 changes were partly reversed within six hours after exposure and fully reversed after 24 hours. Los Angeles subject 7 showed a small but significant loss in FEV_1 with exposure. The other subjects showed no statistically significant physiological changes except in isolated cases among the less stable measurements--thoracic gas volume and total respiratory resistance. These changes were not considered physiologically meaningful. Significant individual changes are summarized in Table 4.

Blood Biochemical Response. Control and post-exposure values for biochemical measures expected to show immediate responses to O_3 or other oxidant challenge are given in Table 5 for the groups. Erythrocyte fragility showed the most striking difference between Canadian and Los Angeles subjects. While both groups showed similar decreases in activity of erythrocyte membrane acetylcholinesterase (a typical response to O_3 exposure¹), only the Canadians showed a significant increase in cell fragility with exposure. This response

was fairly consistent among the four Canadians; no obvious differences in biochemical response between the two highly reactive and the two relatively unreactive subjects were seen (Table 6). The Canadians also showed small but significant increases in serum vitamin E levels, presumably the result of mobilization from body stores in response to the oxidant challenge. A similar increase in the Los Angeles subjects did not attain statistical significance since these individuals were less consistent in baseline values and in response to exposure.

DISCUSSION

These results support the existence of a real difference in reactivity to O_3 between Southern Californians and Canadians studied to date. While the number of subjects tested in the present study is small, the good agreement between present results and previous results in the separate laboratories allows considerably increased confidence in direct comparison of the previous results from Los Angeles and Canada, providing a considerably larger data base from which to judge relative reactivity. Such comparisons are given in Table 7 and in Figure 1. Table 7 gives the mean reactivity in 0.37 ppm O_3 exposures (expressed as loss in FEV_1) of the present subject groups, the entire group of Los Angeles subjects studied to date, and a representative larger group of Montreal subjects. While reactivity is highly variable among different individuals, both the Canadians studied in Montreal and the Canadians in the present study show greater mean reactivity than any Los Angeles group, even a group of relatively sensitive subjects exposed for twice as long as the other groups.⁴ Furthermore, if only the studies done in Los Angeles are considered, without reference to the Canadian studies, the two highly reactive Canadian subjects in the present study were far more reactive than any Los Angeles subject studied under similar conditions--about as reactive at 0.37 ppm as the most sensitive Los Angeles subjects exposed at 0.50 ppm,⁴ and with FEV_1 changes exceeding by three standard deviations the mean for all Los Angeles subjects in equivalent exposures. Comparative dose-response curves for Los Angeles and Montreal subjects, derived by second-order polynomial regression analysis from the

mean response values for each concentration studied, are given in Figure 1. If these curves are adjusted by assuming a consistent difference in O_3 monitoring between the laboratories equal to the maximum observed difference described in the preceding section, they approach each other more closely but still indicate substantially less reactivity in Los Angeles subjects.

If the difference in response to O_3 between Canadians and Southern Californians studied is accepted as real, the hypothesis of adaptation in Southern Californians is supported. Further support of the hypothesis requires demonstration that the subjects tested are meaningfully representative of larger population groups residing in their respective areas, and that identifiable factors other than adaptation are not sufficient to explain the observations. (Positive evidence for O_3 adaptation in humans appears to be lacking at present.)

Subjects in both the Los Angeles and the Canadian studies were predominantly white, middle-class professional and technical workers and pre-professional students, young to middle-aged adults in normal health or with mild respiratory hypersensitivity. While they represent highly selected groups, there appears to be no reason to expect them to be atypical with respect to pulmonary physiological or biochemical status. Neither is there any obvious indication of differences between the Californians and the Canadians in genetic constitution, nutrition, or environmental stresses other than air pollution exposure which could

be the source of their differences in reactivity. Selective migration of O_3 -sensitive individuals away from Los Angeles might be of importance, but no reliable evidence relevant to this possibility exists. Adaptation thus remains the most plausible explanation for the existing data. Since controlled studies designed to demonstrate adaptation in humans are probably impractical, future investigations should seek to define more fully the relative reactivity of populations exposed to different levels of O_3 and the changes in reactivity, if any, exhibited by individuals with little previous exposure who move to O_3 -polluted environments.

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TABLE 1. COMPARATIVE TOTAL OXIDANT (PRIMARILY OZONE) LEVELS FOR SOUTHERN CALIFORNIA AND ONTARIO URBAN AREAS, 1971 (a)

LOCATION	NO. OF MONTHS MONITORED	CONCENTRATIONS (ONE-HOUR AVERAGE), PPM			MEAN
		MAXIMUM	99TH PERCENTILE(b)	90TH PERCENTILE(c)	
Los Angeles area					
Central City	12	.31	.18	.08	.032
Pasadena (d)	12	.68	.31	.14	.052
Toronto area					
Central City	11	.07	.037	.006	.010
Etobicoke (d)	10	.35	.131	.064	.034
Hamilton	10	.25	.124	.070	.042

- NOTES: (a) Los Angeles area values have been multiplied by 1.3 to allow for probable differences in instrument calibration (Reference 10).
 (b) Level exceeded during 1 percent of total hours monitored.
 (c) Level exceeded during 10 percent of total hours monitored.
 (d) Monitoring station showing highest concentration in area during 1971.

SOURCES: California Air Resources Board Ten-Year Summary of Air Quality Data, 1963-1972; Ontario Ministry of the Environment Air Quality Monitoring Report, Vol. 1, 1971.

TABLE 2. SUBJECT CHARACTERISTICS

SUBJECT	SEX	AGE	HT, IN	WT, LB	SMOKING	HISTORY OF:		O ₃ REACTIVITY
						ALLERGY	WHEEZING	
(Canadian Group)								
19	M	39	73	200	N	0	0	++
20	F	33	63	140	S(1)*	0	0	++
21	M	35	69	212	S(15)	+	+	unknown
22	F	32	63	128	S(4)	+	0	unknown
(Los Angeles Group)								
7	M	38	69	155	N	+	+	++
16	M	30	72	175	S(7)	0	0	+
11	M	30	72	155	N	0	0	++
23	F	22	64	107	S(3)	+	+	unknown

* Very light smoker

N = never smoked

S = current cigarette smoker (number in parentheses = approximate lifetime consumption in packs-per-day x years)

0 = none

+

++ = moderate to severe

O₃ reactivity previously documented at 0.75 ppm in Canadian subjects, 0.50 ppm in Los Angeles subjects.

TABLE 3. PHYSIOLOGICAL RESPONSES TO 0.37 PPM O₃ EXPOSURE IN
CANADIAN AND LOS ANGELES GROUPS (MEAN $\pm$ S.D.)

MEASURE	CANADIAN GROUP		LOS ANGELES GROUP	
	CONTROL	EXPOSURE (a)	CONTROL	EXPOSURE (a)
Forced vital capacity (FVC)	4.28 $\pm$ 1.17	4.03 $\pm$ 0.97	4.29 $\pm$ 0.65	4.27 $\pm$ 0.63
Peak expiratory flow ($\dot{V}_{max}$)	10.5 $\pm$ 2.9	9.0 $\pm$ 2.4	10.7 $\pm$ 2.6	11.0 $\pm$ 2.4
Max. exp. flow, 50% FVC ($\dot{V}_{50}$)	4.4 $\pm$ 1.4	3.6 $\pm$ 1.6	4.4 $\pm$ 1.2	4.1 $\pm$ 1.3
Max. exp. flow, 25% FVC ($\dot{V}_{25}$)	1.5 $\pm$ 0.5	1.2 $\pm$ 0.7	1.7 $\pm$ 0.5	1.7 $\pm$ 0.8
1-sec forced exp. volume (FEV ₁)	3.51 $\pm$ 0.92	3.22 $\pm$ 0.84	3.50 $\pm$ 0.56	3.49 $\pm$ 0.55
Total lung capacity (TLC) (b)	5.54 $\pm$ 1.42	5.28 $\pm$ 1.11	5.61 $\pm$ 0.92	5.50 $\pm$ 0.92
Residual volume (RV) (b)	1.16 $\pm$ 0.23	1.18 $\pm$ 0.21	1.11 $\pm$ 0.20	1.13 $\pm$ 0.21
Closing volume, % VC (CV/VC) (b)	10.1 $\pm$ 5.8	10.1 $\pm$ 3.1	10.3 $\pm$ 6.5	9.7 $\pm$ 5.7
Closing capacity, % TLC (CC/TLC) (b)	30.5 $\pm$ 2.2	32.2 $\pm$ 1.4	28.5 $\pm$ 6.4	28.6 $\pm$ 5.5
Delta nitrogen (ΔN_2 /liter) (b)	1.1 $\pm$ 0.7	1.3 $\pm$ 0.6	0.9 $\pm$ 0.5	1.2 $\pm$ 0.8
Functional residual capacity (FRC) (c)	2.70 $\pm$ 0.38	2.96 $\pm$ 0.32	2.97 $\pm$ 0.42	3.11 $\pm$ 0.30
Airway resistance (Raw) at FRC (c)	1.50 $\pm$ 0.44	1.76 $\pm$ 0.40	1.40 $\pm$ 0.63	1.27 $\pm$ 0.47
Total lung capacity (c)	5.97 $\pm$ 1.21	6.19 $\pm$ 1.19	6.26 $\pm$ 1.00	6.44 $\pm$ 0.75
Residual volume (c)	1.36 $\pm$ 0.34	1.77 $\pm$ 0.20	1.52 $\pm$ 0.42	1.78 $\pm$ 0.23

TABLE 3. PHYSIOLOGICAL RESPONSES TO 0.37 PPM O₃ EXPOSURE IN
CANADIAN AND LOS ANGELES GROUPS (MEAN $\pm$ S.D.) (continued)

<u>MEASURE</u>	<u>CANADIAN GROUP</u>		<u>LOS ANGELES GROUP</u>	
	<u>CONTROL</u>	<u>EXPOSURE (a)</u>	<u>CONTROL</u>	<u>EXPOSURE (a)</u>
Total respiratory resistance (d)				
3 hertz	7.5 $\pm$ 3.8	7.3 $\pm$ 1.5	5.41 $\pm$ 1.1	5.0 $\pm$ 1.1
6 hertz	6.0 $\pm$ 0.6	6.1 $\pm$ 0.9	5.0 $\pm$ 2.3	5.3 $\pm$ 2.4
9 hertz	6.3 $\pm$ 2.1	5.3 $\pm$ 1.1	4.8 $\pm$ 1.7	5.0 $\pm$ 1.1
12 hertz	5.2 $\pm$ 1.3	4.8 $\pm$ 0.9	4.5 $\pm$ 1.8	4.2 $\pm$ 1.2

NOTES: (a) All changes with exposure are non-significant ($P > .05$ for one-way analysis of variance)

(b) Single-breath nitrogen method

(c) Pressure plethysmograph method

(d) Forced oscillation method

TABLE 4. SIGNIFICANT INDIVIDUAL CHANGES IN FORCED-EXPIRATORY AND SINGLE-BREATH NITROGEN MEASURES WITH EXPOSURE

	CONTROL	EXPOSURE
SUBJECT 7 (LOS ANGELES)		
FEV ₁ (best of 3 trials)	3.82	3.70
FEV ₁ (mean)	3.80 ± .02	3.65 ± .05
SUBJECT 19 (CANADIAN)		
FVC (best of 3 trials)	6.07	4.90
FVC (mean)	6.00 ± .05	4.85 ± .08*
FEV ₁ (best of 3 trials)	4.81	4.20
FEV ₁ (mean)	4.74 ± .06	3.80 ± .56
TLC (mean)	7.62 ± .05	6.40 ± .49
ΔN ₂ (mean)	0.53 ± .06	1.25 ± .35
SUBJECT 20 (CANADIAN)		
FVC (best of 3 trials)	3.26	3.00
FVC (mean)	3.24 ± .03	2.92 ± .09*
FEV ₁ (best of 3 trials)	2.54	2.25
FEV ₁ (mean)	2.52 ± .04	2.17 ± .07
TLC (mean)	4.25 ± .03	3.98 ± .05*

* Mean change significant, $P < .01$, by unpaired t test. All other mean changes significant, $P < .05$. Three measures obtained under each condition.

TABLE 5

BLOOD BIOCHEMICAL RESPONSE OF CANADIANS
VS. SOUTHERN CALIFORNIANS--GROUP DATA, MEAN $\pm$ S.D.

ANALYSIS, GROUP	CONTROL	EXPOSURE	N	T	P
Erythrocyte Fragility (a)					
Canadian (b)	15.3 $\pm$ 1.2	22.1 $\pm$ 2.2	4	9.53	<.005
Los Angeles (b)	18.6 $\pm$ 1.9	17.5 $\pm$ 1.3	4	0.48	N.S.
Total L.A. (c)	16.8 $\pm$ 2.4	17.3 $\pm$ 3.2	13	0.97	N.S.
Erythrocyte Acetylcholinesterase Activity (d)					
Canadian	22.3 $\pm$ 1.4	19.9 $\pm$ 1.9	4	8.72	<.005
Los Angeles	21.0 $\pm$ 1.0	19.4 $\pm$ 1.0	4	7.83	<.005
Total L.A.	22.3 $\pm$ 2.9	20.6 $\pm$ 2.4	13	5.49	<.01
Erythrocyte Glucose-6-Phosphate Dehydrogenase Activity (d)					
Canadian	6.42 $\pm$ 0.47	6.84 $\pm$ 0.80	4	2.46	N.S.
Los Angeles	7.02 $\pm$ 0.30	7.13 $\pm$ 0.77	4	0.32	N.S.
Total L.A.	4.50 $\pm$ 1.02	4.81 $\pm$ 1.21	13	2.18	<.05
Erythrocyte Glutathione (e)					
Canadian	30.9 $\pm$ 5.5	30.4 $\pm$ 5.4	4	2.96	N.S.
Los Angeles	31.4 $\pm$ 3.9	33.2 $\pm$ 2.0	4	2.00	N.S.
Total L.A.	31.6 $\pm$ 4.7	28.2 $\pm$ 3.4	12	2.72	<.025
Serum Lipid Peroxidation (f)					
Canadian	.219 $\pm$.022	.242 $\pm$.030	4	1.00	N.S.
Los Angeles	.300 $\pm$.032	.307 $\pm$.015	4	1.80	N.S.
Total L.A.	.191 $\pm$.060	.199 $\pm$.030	13	0.62	N.S.
Serum Vitam E (g)					
Canadian	1.71 $\pm$.34	1.83 $\pm$.33	4	4.10	<.025
Los Angeles	2.36 $\pm$ 1.65	2.50 $\pm$ 1.61	4	1.28	N.S.
Total L.A.	2.05 $\pm$.98	2.15 $\pm$.98	13	0.83	N.S.

NOTES: (a) Percent hemolysis in 2 percent hydrogen peroxide. (b) Present study. (c) All Los Angeles subjects under initial exposure to 0.37 ppm O₃ studied to date, including present Los Angeles subjects. (d) μ mol substrate consumed/g hemoglobin/min. (e) mg/100 ml. (f) Equivalent μ g malonaldehyde/ml. (g) μ g/ml.

TABLE 6
INDIVIDUAL BLOOD BIOCHEMICAL
RESPONSES OF CANADIAN SUBJECTS

<u>ANALYSIS, SUBJECT</u>	<u>CONTROL</u>	<u>EXPOSURE</u>	<u>% CHANGE</u>
Erythrocyte Fragility			
19 (R)	14.2	21.9	+53
20 (R)	14.4	19.3	+34
21	16.7	24.9	+49
22	15.8	22.5	+42
Erythrocyte Acetylcholinesterase Activity			
19 (R)	20.8	18.2	-12
20 (R)	21.7	18.5	-15
21	24.0	22.3	- 7
22	22.9	20.7	-10
Serum Vitamin E			
19 (R)	1.73	1.90	+10
20 (R)	1.94	1.98	+ 2
21	1.94	2.09	+ 8
22	1.22	1.34	+10

NOTE: (R) = Clinically and physiologically reactive subject. Units are given in Table 5.

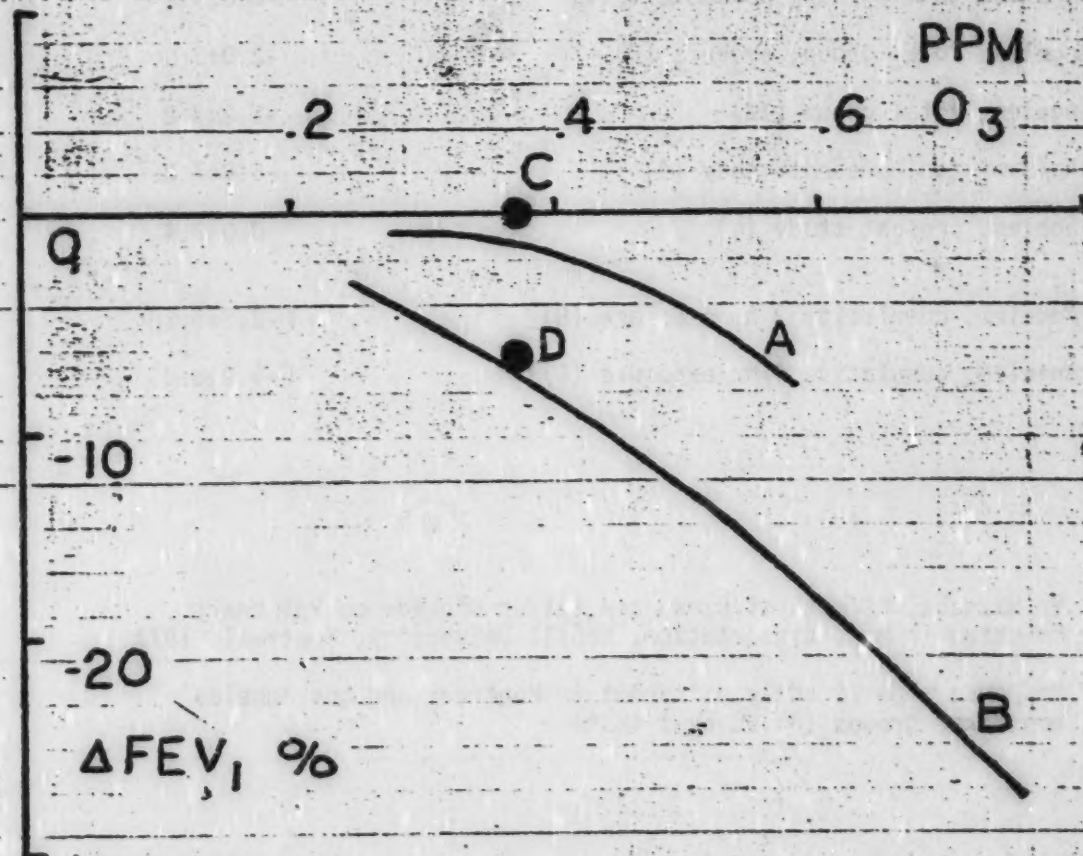
TABLE 7. MEAN REACTIVITY OF SUBJECT GROUPS UNDER INITIAL EXPOSURE TO 0.37 PPM O₃ FOR 2 HOURS WITH INTERMITTENT LIGHT EXERCISE

GROUP (NUMBER OF SUBJECTS)	PERCENT CHANGE IN FEV ₁ , MEAN±S.D.
Montreal, nonsmokers (6)*	-7.4±5.6**
Montreal, smokers (6)*	-3.9±1.7
Los Angeles, total group, nonsmokers (7)	-0.6±3.4**
Los Angeles, total group, smokers (6)	-2.0±3.0
Los Angeles, total group (13)	-1.2±3.2
Toronto/Hamilton, present study (4)	-5.8±7.3
Los Angeles, present study (4)	0.0±2.4
[Los Angeles, cumulative 2-hr exposure (8)]	[-2.5±3.2]
[Los Angeles, cumulative 4-hr exposure (4)]	[-4.0±8.3]

* M. Hazucha, Effects of Ozone and Sulfur Dioxide on Pulmonary Function in Man, dissertation, McGill University, Montreal, 1973.

** Response significantly different in Montreal and Los Angeles nonsmoker groups (P<.05 by t test).

FIGURE 1.
COMPARATIVE DOSE-RESPONSE BEHAVIOR



LEGENDS

FIGURE 1. COMPARATIVE DOSE-RESPONSE BEHAVIOR. Curve A--mean response (percent loss in FEV_1) of previously-studied Southern Californians exposed at 0.25-0.50 ppm O_3 . Curve B--mean response of previously-studied Canadians exposed at 0.25-0.75 ppm (reference 7). Point C--mean response of Southern Californians in present study. Point D--mean response of Canadians in present study. Curves determined by second-order regression analysis. All exposures for 2 hours with intermittent light exercise.

**STUDIES IN ADAPTATION TO AMBIENT OXIDANT AIR POLLUTION:
Effects of Ozone Exposure in Los Angeles Residents vs.
New Arrivals**

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STUDIES IN ADAPTATION TO AMBIENT OXIDANT AIR POLLUTION:
Effects of Ozone Exposure in Los Angeles Residents vs.
New Arrivals

ABSTRACT

To test the hypothesis that adaptation protecting against acute effects of ambient ozone (O_3) exposures develops in Los Angeles residents, human volunteers were exposed to 0.4 ppm O_3 under conditions simulating ambient pollution exposures. Blood biochemical, pulmonary physiological, and clinical responses were assessed. Los Angeles residents (N=6) showed only minimal clinical or physiological response to O_3 , while new arrivals (N=9) showed significant losses in pulmonary function and a tendency toward increased symptoms. Most biochemical responses did not differ significantly between residents and new arrivals. These results agree with others in suggesting that exposures to elevated ambient concentrations of O_3 produce adaptation in at least some residents of photochemical pollution areas. The underlying mechanisms and long-term consequences of such adaptation are unknown.

INTRODUCTION

Development of tolerance to ozone (O_3) and other irritant gases in experimental animals was first described by Stokinger and co-workers approximately 20 years ago (1) and has been studied extensively since. The subject has been reviewed by Fairchild (2) and Morrow (3). Salient features of animal tolerance include the following: (a) Pretreatment with a relatively low O_3 dose will prevent death or severe lung injury which would otherwise occur with a higher dose. (b) This tolerance gradually disappears after cessation of O_3 exposure. (c) Cross tolerance exists among O_3 and other irritant gases, including some which, like O_3 , are powerful oxidizing agents and others which are not. (d) Tolerance does not prevent the development of chronic lung lesions following repeated exposures. (e) Tolerance results in decreased edema formation in response to O_3 challenge, but no diminution of cytotoxic effects of O_3 is observable (4). (f) The biological mechanisms responsible for tolerance are largely unknown.

The observation that animals can respond to a toxic inhalation challenge in a manner which prevents some of the short-term adverse effects of further exposures suggests the possibility that an analogous response might occur in humans exposed to community air pollution. We use the term "adaptation" to describe this hypothetical response in humans, since the doses of toxicants being considered are much less than in animal "tolerance" studies, and since responses are less severe and

perhaps depend on different biological phenomena. Metropolitan Los Angeles experiences uncommonly high ambient levels of O_3 and other oxidants during photochemical smog episodes, thus residents of this area constitute an attractive group in which to investigate the possibility of adaptation. That Los Angeles residents suffer less deleterious effects of ambient exposures than visitors to the area has been previously suggested (5), but the hypothesis has never been tested extensively.

Previous work in our laboratory (6,7) showed that some healthy Los Angeles residents develop respiratory symptoms and function changes when exposed to O_3 concentrations of 0.37-0.50 ppm--less than maximum ambient concentrations in the area. Similar studies in Canadians not frequently exposed to ambient oxidants (8,9) appeared to show a greater mean effect of a given dose, suggesting that responses in Los Angeles residents might have been reduced by adaptation. Methodological differences between studies might have explained the apparently different responses, however. To test this possibility, a cooperative investigation was undertaken to compare experimental methods and responses of a small sample of subjects to 0.4 ppm O_3 (10). The results reproduced to a great extent the previous finding of less reactivity in Los Angeles residents as compared to Canadians and failed to reveal any methodological factors which could account for this difference. The hypothesis of adaptation was thus supported. To test the hypothesis more rigorously, the present study was undertaken in

order to compare the effects of 0.4 ppm O_3 in somewhat larger and more carefully matched groups of Los Angeles residents and non-residents.

METHODS

The null hypothesis tested was as follows: Healthy Los Angeles residents (three years or more in area) and new arrivals (five days or less in area) will not differ in mean clinical, physiological or biochemical response to 0.4 ppm O_3 exposure under conditions simulating ambient pollution episodes. Rejection of the null hypothesis with new arrivals showing significantly greater mean response would be the necessary result if the hypothesis of O_3 adaptation in residents were to be supported.

The exposure facility and basic experimental design have been described in detail previously (11). Volunteer subjects were studied on two successive days. The first day's exposure was to purified air only; the second day's exposure was to 0.40 ppm O_3 in purified air. Exposures lasted 2 hr 15 min. During the first two hours, each subject exercised at a workload sufficient to increase minute volume to approximately twice the resting level (150-200 kg-m/min) for 15 min in every half hour. During the last 15 min pulmonary function tests were performed; these included forced vital capacity (FVC), one-second forced expiratory volume (FEV_1), maximum midexpiratory flow rate (MMF), total respiratory resistance by forced oscillation (R_t), and indices of the single-breath nitrogen test: closing volume as a

percent of vital capacity (CV/VC), delta nitrogen or slope of the alveolar plateau (ΔN_2). Each subject's test results were expressed as control values (those obtained after purified-air exposure) and as O_3 responses (differences between post- O_3 exposure and control values). Subjects' symptoms during and following exposure were recorded and scored semiquantitatively according to severity and duration using a standard interview questionnaire administered by the project medical officer. The symptom response to O_3 was expressed as the difference in symptom score between O_3 exposure and control days. Venous blood samples were drawn immediately following exposure, and erythrocyte (RBC) and serum analyses were performed to detect changes expected to result from an oxidant challenge, as described previously (12).

Paired statistical tests with each subject serving as his own control were applied to detect differences between control and O_3 conditions for the resident group and for the new-arrival group. Unpaired tests were applied to compare between groups. For physiological measures, only O_3 responses were compared between groups, as control values were expected to depend mostly on body size and not on adaptation. For biochemical measures, control values could have differed between groups as a consequence of adaptation, therefore both control values and O_3 responses were compared statistically. In addition to the commonly employed t tests, analogous nonparametric tests--the Wilcoxon signed-rank test for paired analyses and the Mann-Whitney U test for between-group analyses--were applied to the pulmonary function data. The nonparametric tests were expected to be possibly more powerful in analyzing these data since the data were expected to be skewed, whereas t tests require a normal

distribution for greatest reliability. Skewness is inherent in data of this nature since there is considerable variability between individuals in reactivity to exposure, and since function measures remain similar to control values in relatively unreactive subjects but deviate from control values in only one direction in more reactive subjects. Symptom data, which were not rigorously quantitative and not necessarily expected to show a normal distribution even under control conditions, were analyzed only with the nonparametric tests.

Subjects were recruited within the incoming 1975 class of the USC School of Physical Therapy. Fifteen of a possible 44 individuals volunteered to be studied; six of these were metropolitan Los Angeles residents and nine were nonresidents. Studies were conducted during September, i.e. late in the summer smog season when residents should have had ample time to develop adaptation. Nonresidents were studied within five days of their arrival in Los Angeles; they were instructed to minimize intercurrent ambient oxidant exposures by remaining in coastal areas of metropolitan Los Angeles and/or remaining indoors and at rest during peak oxidant hours.

Individual subject characteristics are given in Table 1. Since the nonresidents included two males, while the residents were all female, the possible effect of sex differences on the overall results was examined. The males' data were compared individually with the female nonresidents' for the three measures which showed significant ($P < .05$) group differences. Both males' values fell within the females' range, except that one male had the largest control and post-exposure FEV_1 . When statistical analyses were repeated excluding the males' data, mean group responses were actually

larger than when the males were included; however, due to the reduction in sample size the level of significance of the group differences decreased-- $.05 < P < .10$ with males excluded. Overall, no evidence was found that sex differences affected the results; this was also the case in the previous study (10).

RESULTS

Individual physiological and clinical responses are given qualitatively in Table 1, and group mean physiological and symptom measures are summarized in Table 2. The residents as a group showed no significant O_3 responses except for slight decrease in ΔN_2 .

TABLE 1. INDIVIDUAL SUBJECT CHARACTERISTICS

<u>I.D. NO.</u>	<u>SEX</u>	<u>AGE, YEARS</u>	<u>HT., INCHES</u>	<u>WT. POUNDS</u>	<u>SMOKING</u>	<u>YEARS IN LOS ANGELES AREA</u>	<u>O₃ RESPONSE*</u>
LOS ANGELES RESIDENTS							
52	F	22	69	158	current	18	-
59	F	25	68	118	-	3	P
60	F	25	68	118	former	18	S
65	F	21	67	138	-	10	P
66	F	25	63	94	-	3	-
69	F	22	65	125	-	14	-
NONRESIDENTS (NEW ARRIVALS)							
47	F	22	68	140	-	-	P,S
49	M	22	71	160	-	-	P,S
50	F	21	66	115	-	†	-
51	F	21	62	125	-	-	P,S
53	F	22	73	155	-	-	-
55	F	22	68	125	-	-	S
56	F	23	62	120	-	-	P,S
57	F	21	64	121	-	-	P
58	M	24	72	170	current	-	P

* P = physiological response--significant ($P < .05$) loss in FVC and/or FEV_1 with O₃ exposure relative to control, determined by t test, 3 measurements under each condition. S = symptom response--increase in symptom score of ≥ 4 units (arbitrary definition of "clinically significant" response).

† Spent previous summer in area.

TABLE 2. COMPARATIVE PULMONARY FUNCTION AND SYMPTOM MEASURES--
CONTROL VALUES AND CHANGE WITH O₃ EXPOSURE, MEAN $\pm$ S.D.

	RESIDENTS	NEW ARRIVALS	INTERGROUP COMPARISON	
			t	U
Control FVC, liters	4.01 $\pm$.40	4.57 $\pm$.89		
FVC change, liters	-.093 $\pm$.155(a)	-.164 $\pm$.202(b)	0.72(a)	20(a)
Control FEV ₁ , liters	3.49 $\pm$.22	3.84 $\pm$.49		
FEV ₁ change, liters	-.018 $\pm$.098(a)	-.171 $\pm$.174(b)	1.93(a)	9(P<.05)
Control MMF, l/sec	4.06 $\pm$.70	4.23 $\pm$.86		
MMF change, l/sec	+.175 $\pm$.336(a)	-.252 $\pm$.320(b)	2.48(P<.05)	9.5(P<.05)
Control CV/VC%	7.6 $\pm$ 5.5	6.8 $\pm$ 5.8		
CV/VC% change	+0.4 $\pm$ 2.8(a)	+0.5 $\pm$ 3.2(a)	0.12(a)	24(a)
Control Δ N ₂ , %N ₂ /l	0.95 $\pm$.15	0.93 $\pm$.23		
Δ N ₂ change, %N ₂ /l	-.117 $\pm$.094(c)	-.050 $\pm$.206(a)	0.73(a)	21.5(a)
Control R _t , cmH ₂ O/(l/sec)	4.02 $\pm$.99	3.25 $\pm$.90		
R _t change, cmH ₂ O/(l/sec)	+0.13 $\pm$.98(a)	+0.20 $\pm$.45(a)	0.18(a)	17(a)
Control symptom score	4.9 $\pm$ 5.1	3.6 $\pm$ 3.5		
Symptom score change	+0.2 $\pm$ 5.5(a)	+2.7 $\pm$ 4.8(a)	-	19(a)

- NOTES: (a) Not significant
 (b) Significant decrement after exposure, P<.05 by paired t test and by Wilcoxon signed-rank test.
 (c) Change not significant by signed-rank test; apparent "improvement" after exposure according to paired t test (P<.05).

Increases in ΔN_2 are normally expected in chronic pulmonary dysfunction and in acute responses to O_3 exposure (6). Decreased values represent increased uniformity of ventilation distribution and thus could be considered an improvement in function. On the other hand, more uniform distribution could be the result of adverse physiological changes, such as complete "closure" of a few small airways previously only partially obstructed. Nonresidents showed a smaller, nonsignificant decrease in ΔN_2 , but showed significant O_3 responses in FVC, FEV_1 , and MMF. The MMF response was significantly more severe than in the residents according to the intergroup comparison, but the FVC responses did not differ significantly between the groups. The FEV_1 loss was significantly more severe in nonresidents than in residents according to the U test ($P=.03$), but not according to the t test ($P=.06$). Since the distributions of FEV_1 responses appear skewed (Figure 1), the results of the U test may be more reliable. Neither group showed significant responses of CV/VC, R_t , or symptom score, but the nonresidents showed a trend toward increased symptom score with O_3 exposure.

Group mean biochemical measurements and significant changes related to exposure are summarized in Table 3. None of the analyses showed significant differences in control values between residents and new arrivals, although residents showed trends toward less fragility of RBCs as determined by hydrogen peroxide challenge, and higher serum concentrations of Vitamin E. Both groups showed O_3

TABLE 3. COMPARATIVE BIOCHEMICAL MEASURES--CONTROL VALUES AND CHANGE WITH O₃ EXPOSURE, MEAN $\pm$ S.D.

		<u>Residents</u>	<u>New Arrivals</u>	<u>Intergroup Comparison</u> <u>t</u>
RBC fragility (% hemolysis in H ₂ O ₂)	Control	23.0 $\pm$ 15.2	31.4 $\pm$ 8.0	1.39 (a)
	Change	+6.2 $\pm$ 5.5 (b)	+3.1 $\pm$ 3.4 (b)	1.35 (a)
RBC acetylcholinesterase mM/ml/min)	Control	17.5 $\pm$ 1.8	18.8 $\pm$ 1.9	1.31 (a)
	Change	-0.6 $\pm$ 0.6 (c)	-0.8 $\pm$ 0.5 (d)	0.83 (a)
RBC glutathione, (mg %)	Control	33.3 $\pm$ 6.2	35.3 $\pm$ 5.0	0.67 (a)
	Change	-1.6 $\pm$ 1.9 (a)	-2.0 $\pm$ 3.0 (a)	0.28 (a)
RBC 2,3-diphospho- glycerate (μ M/g Hb)	Control	14.9 $\pm$ 1.6	14.7 $\pm$ 4.1	0.12 (a)
	Change	+1.7 $\pm$ 0.7 (d)	+1.0 $\pm$ 3.3 (a)	0.50 (a)
RBC glucose-6-phosphate dehydrogenase (U/g Hb/min)	Control	5.22 $\pm$ 1.14	5.05 $\pm$ 0.86	0.33 (a)
	Change	+0.23 $\pm$ 0.43 (a)	+0.21 $\pm$ 0.32 (a)	0.09 (a)
RBC lactate dehydrogenase (U/g Hb/min)	Control	107 $\pm$ 16	112 $\pm$ 15	0.59 (a)
	Change	-6.6 $\pm$ 12.4 (a)	+8.9 $\pm$ 9.5 (d)	2.74 (e)
RBC glutathione peroxidase (U/ml/min)	Control	8.6 $\pm$ 1.8	8.8 $\pm$ 1.7	0.23 (a)
	Change	+0.8 $\pm$ 1.1 (a)	+0.3 $\pm$ 0.8 (a)	1.11 (a)
Serum Vitamin E (mg %)	Control	2.77 $\pm$ 0.79	2.64 $\pm$ 0.38	0.44 (a)
	Change	+0.09 $\pm$ 0.15 (a)	+0.03 $\pm$ 0.14 (a)	0.66 (a)
Serum glutathione reductase (mU/ml/min)	Control	23.7 $\pm$ 4.2	22.6 $\pm$ 3.0	0.60 (a)
	Change	+1.5 $\pm$ 1.7 (a)	+2.8 $\pm$ 3.0 (d)	0.98 (a)

- NOTES: (a) Not significant
 (b) Significant ($P < .05$) change after exposure by t test; not significant ($.05 < P < .10$) by signed-rank test.
 (c) Significant ($P < .05$) change after exposure by signed-rank test; not significant ($.05 < P < .10$) by t test.
 (d) Significant ($P < .05$) change after exposure by t test and by signed-rank test.
 (e) Significant difference between groups ($P < .05$).
 (f) Intergroup comparisons by U test gave same results as t test in every case, with respect to significance at .05 level.

responses generally similar to those seen previously (12)--increased RBC fragility, reduced RBC acetylcholinesterase activity, and tendencies toward increased activity of pentose pathway enzymes (which would tend to protect against excessive oxidation of cellular components). Lactate dehydrogenase (LDH) activity was the only biochemical measure to show a significant difference between groups in response to O_3 . New arrivals showed the expected increase in LDH activity, while residents showed a decrease, in contrast to previous findings (12). The biological significance of this observation, if indeed it represents other than a chance occurrence, is unclear.

DISCUSSION

These results support the hypothesis of adaptation to O_3 in Los Angeles residents. Statistical differences found between residents and new arrivals are relatively small, as should be expected given the unavoidably small sample sizes and the typically large individual variability in O_3 responses. Controlled-exposure studies cannot be done on a large enough scale to conclusively establish differences in response between populations, but the essential agreement of present and previous results in small-scale studies considerably strengthens the case for the existence of such differences. Various factors unrelated to inherent adaptive biological responses could explain these results--selective migration or diet, for example (10). No such factor has yet been identified, leaving adaptation as the most plausible explanation for the experimental observations. No biochemical

index of the adapted state has yet been found in animals or in man, nor are the physiological and biochemical mechanisms of O_3 toxicity well understood. Further investigations in these areas will be necessary before the biological mechanisms of the adaptive response (if it exists) can be elucidated. Of particular interest is the possibility that adaptive mechanisms may be inoperative in certain individuals, who might then be at increased risk of developing chronic respiratory disease.

The phenomenon of adaptation may ultimately, but should not presently, be taken into account in setting ambient or occupational air-quality standards. By analogy with animal studies, it appears that human adaptation to acute O_3 effects might not protect against the possible development of chronic lung damage after many exposures. Unless this possibility and the possibility of failure of adaptation are conclusively ruled out, air quality standards should continue to be set to protect the susceptible, least well-adapted individuals in the exposed population.

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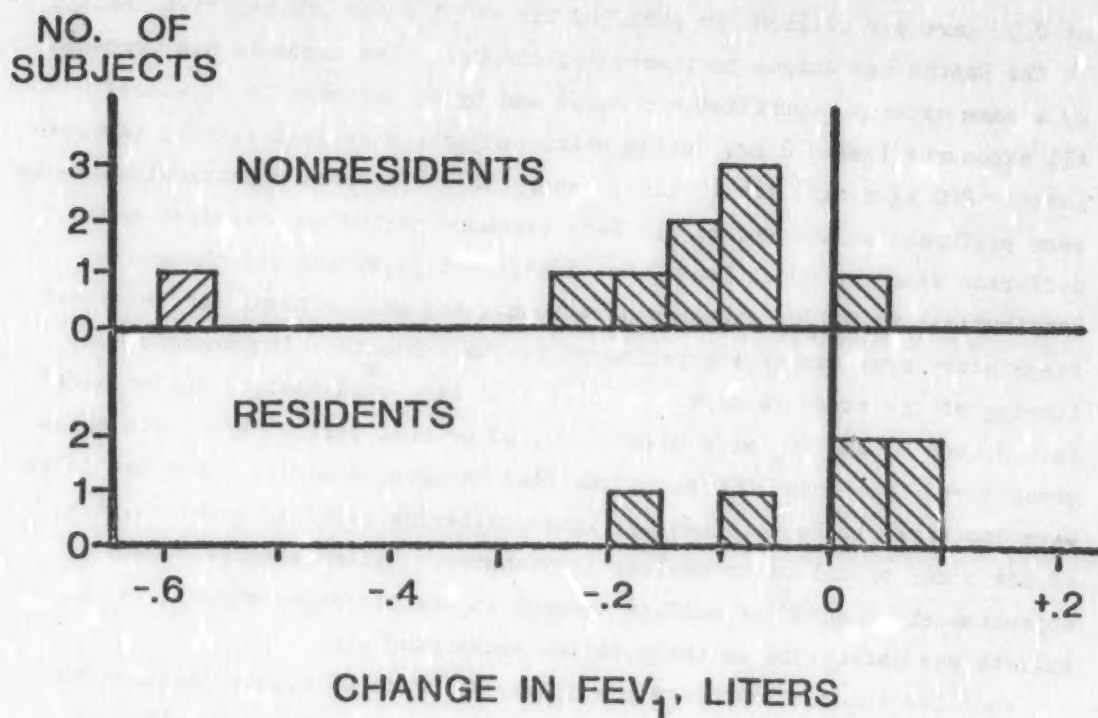


FIGURE 1. Histograms of O₃ responses in FEV₁ (change between post-exposure and control measurements) for non-residents and residents -- number of subjects showing a given response (within a 0.5-liter interval) vs. magnitude of response.

1. RESPIRATORY EFFECTS OF EXPOSURE TO OZONE-SULFUR DIOXIDE MIXTURES

SCOR-Environmental Health Service

Summary

Four normal Los Angeles residents were exposed to O_3 plus SO_2 , each gas at 0.37 part per million, in purified air at $31^\circ C$ and 35% relative humidity in the Rancho Los Amigos environmental chamber. The exposure was preceded by a sham exposure (purified air only) and by an exposure to O_3 alone. All exposures lasted 2 hr, during which subjects exercised lightly (approximately 200 kg-m/min) for 15 min in every 30. Pulmonary physiological tests were performed at the end of the 2-hr exposure period and compared among different exposure conditions. No significant physiological changes attributable to O_3/SO_2 exposure were found, and only minimal and transient respiratory symptoms were experienced by the subjects. Atmospheric monitoring of the exposure chamber showed that many condensation nuclei were formed when O_3 and SO_2 were mixed (100,000 or more particles/ml with gases present vs. less than 1000/ml in purified background air). These particles were too small to be detected by light-scattering particle counters, i.e. of the order of 0.1 μm or smaller in diameter. Filter samples showed approximately 1 $\mu g/m^3$ of sulfate aerosol in the mixed-gas atmosphere; no sulfate was detectable in the purified background air.

Four Los Angeles residents previously found to be highly reactive to O_3 exposure were exposed in the same manner as the normal subjects. Of these, two showed no more than minimal pulmonary function changes with O_3/SO_2 exposure and two showed mild but significant losses in forced-expiratory measures. In the most reactive subject, these changes were greater than previously observed in a comparable exposure to O_3 alone on two successive days.

As the above results suggested much less marked short-term toxicity of O_3/SO_2 than previously reported by Hazucha et al. (Fed. Proc. 33: 350, 1974) in normal Canadians, a cooperative study was initiated to investigate the source of the difference between the two studies. Four previously-studied Canadian subjects were brought to this laboratory and exposed for 2 hr with intermittent light exercise to purified air on one day and to 0.37 ppm O_3 plus 0.37 ppm SO_2 on the following day. Temperature was $21^\circ C$ and relative humidity 50%, giving comparability to the previous Canadian

exposure conditions. One subject experienced a reaction of severity comparable to or greater than that previously experienced in the Canadian study. Two others reacted much less severely in this laboratory than in Canada, and the remaining subject showed a relatively mild reaction in both places. Pulmonary-function changes for the group in the present study were not statistically significant due to the large individual variation. Particulate formation from the O_3/SO_2 mixture occurred in this study in essentially the same manner as described for the previous studies.

The four reactive Los Angeles residents previously mentioned, plus one additional O_3 -reactive Los Angeles subject, were exposed under the conditions employed for the Canadian subjects. Results were similar to those previously obtained in these individuals. Pulmonary function changes seen were slight, but were more consistent than in the Canadians, allowing the mean loss in one-second forced expiratory volume to attain marginal statistical significance. Mean function changes were smaller in these subjects than in the Canadians but larger than observed previously in these subjects under exposure to O_3 alone. Symptoms were also less severe than in the Canadians but more severe than in the same subjects when exposed to O_3 . Typical symptoms in both groups were cough and substernal discomfort; some subjects also reported a burning sensation on the skin, not noticed in O_3 exposures.

Conclusions

The Canadians studied in this laboratory appeared to react less than they had previously in Canada in presumably similar O_3/SO_2 exposures. They also appeared to react more severely than Los Angeles residents. These findings are consistent with the possibility of biological adaptation to chronic ambient pollution exposure in Los Angeles residents. More study of interactions of O_3 , SO_2 , and particulates is required, since such interactions are apparently inevitable in controlled exposures as well as in polluted ambient air and may grossly change the manner in which the pollutants impinge upon the respiratory tract. Different background particulate concentrations in the present studies and the Canadian studies may be the source of the difference in subject responses. Particulate levels were probably higher in the Canadian studies, since less elaborate air filtration was available.

Data

PULMONARY FUNCTION DATA FOR LOS ANGELES GROUPS EXPOSED TO O_3/SO_2 AT $31^\circ C$
MEAN, (STANDARD DEVIATION)

	EXPOSURE: <u>Sham</u>	<u>O_3</u>	<u>O_3/SO_2</u>
"Normal" Group (N = 4)			
FVC	4.80 (.56)	4.73 (.54)	4.66 (.59)
FEV ₁	3.85 (.54)	3.91 (.59)	3.76 (.64)
$\dot{V}_{max}$ 50% FVC	5.5 (0.7)	5.6 (1.1)	5.1 (1.0)
CV (% VC)	13.2 (4.3)	13.8 (4.2)	13.4 (5.3)
Delta N ₂	.70 (.18)	.71 (.24)	.69 (.24)
"Reactive" Group (N = 4)			
FVC	5.11 (.89)	5.06 (1.02)	4.90 (1.05)
FEV ₁	4.19 (.60)	4.11 (.70)	3.96 (.74) (P < .05)
$\dot{V}_{max}$ 50% FVC	5.0 (0.8)	4.8 (0.7)	4.6 (0.6)
CV (% VC)	11.6 (4.3)	10.6 (5.2)	11.4 (4.0)
Delta N ₂	.61 (.06)	.65 (.04)	.76 (.13) (P < .05)

Note: Volumes in liters, flows in liters/second, delta nitrogen in percent N₂ concentration increase per liter expired. Changes not significant at .05 level unless indicated otherwise.

PULMONARY FUNCTION DATA FOR CANADIAN & LOS ANGELES GROUPS EXPOSED AT $21^\circ C$
MEAN, (STANDARD DEVIATION)

	<u>Sham</u>	<u>O_3/SO_2</u>
Canadian Group (N = 4)		
FVC	5.83 (.67)	5.64 (.75)
FEV ₁	4.60 (.91)	4.35 (1.02)
$\dot{V}_{max}$ 50% FVC	5.6 (2.4)	4.7 (2.4)
CV (% VC)	6.0 (0.6)	5.5 (1.8)
Delta N ₂	.78 (.27)	.85 (.10)
"Reactive" Los Angeles Group (N = 5)		
FVC	4.62 (.99)	4.50 (.98)
FEV ₁	3.72 (.80)	3.59 (.79) (P .05)
$\dot{V}_{max}$ 50% FVC	4.3 (1.1)	4.0 (0.8)
CV (% VC)	9.4 (5.2)	9.8 (3.5)
Delta N ₂	.93 (.38)	1.02 (.50)

(See notes following preceding table)

INDIVIDUALS' RESPONSES IN PREVIOUS CANADIAN STUDY VS. PRESENT
(LOS ANGELES) STUDY--PERCENT CHANGE FROM CONTROL VALUES

MEASURE:	FVC		FEV ₁	
STUDY:	<u>Can.</u>	<u>L.A.</u>	<u>Can.</u>	<u>L.A.</u>
Subject No. 31	-2	-6	-9	-13
32	-51	0	-58	-2
33	-5	-2	-1	-6
34	-29	-3	-30	-2
(Mean)	(-22)	(-3)	(-24)	(-6)

NITROGEN DIOXIDE INHALATION AND HUMAN BLOOD BIOCHEMISTRY

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NITROGEN DIOXIDE INHALATION AND HUMAN BLOOD BIOCHEMISTRY

ABSTRACT

Blood from ten young adult male humans, exposed to 1.0 ppm or 2 ppm nitrogen dioxide (NO_2) for 2.5-3.0 hours, was examined for evidence of biochemical changes. The experiments lasted three days. The subjects entered an environmental chamber, performed mild exercise and completed a series of pulmonary physiology measurements while breathing filtered air. Blood samples were then taken and analyzed. This regimen was repeated on the second and third day except the chamber atmosphere now contained 1.0 ppm or 2 ppm NO_2 . Paired group analyses were performed on the data. A statistically significant decrease was observed in the activity of the erythrocyte (RBC) membrane enzyme acetylcholinesterase (AcChase) at both NO_2 levels. Levels of thiobarbituric acid reactive substances were elevated following inhalation of both levels of the irritant gas but the differences were statistically significant only at the higher concentration. Other biochemical values were variable but suggested the possibility of a transitory response to oxidation damage resulting from the NO_2 inhalation.

INTRODUCTION

The toxic nature of very high levels of nitrogen dioxide (NO_2) has been known at least as long as man has been using silos for wet storage of livestock food. This knowledge has stimulated man's curiosity about the mechanism of toxicity and many experiments have been performed with laboratory animals in an attempt to elucidate the structural and functional changes resulting from acute and chronic exposure to the gas. Early experiments were relatively crude because methods for generating and monitoring the gas were not sufficiently developed.¹ With better methods the experiments have become more sophisticated and much more useful information began to emerge.² Nitrogen dioxide is commonly found in urban and industrial atmospheres but at much lower levels than were employed in many early experiments. More recently, experiments have been designed in which laboratory animals were exposed to levels of NO_2 found commonly in outside atmospheres. Biological effects have been noted at these levels. Typical experiments, for example, have shown hypertrophy and hyperplasia of alveolar cells after three weeks continuous exposure to 2 ppm NO_2 ,³ a proteinuria in guinea pigs following 14 days exposure to 0.5 ppm NO_2 ,⁴ and a decrease in the animal's resistance to bacterial infection.⁵ Reports of significant biochemical changes in blood after *in vivo* exposures to low levels of NO_2 have not come to our attention. We have exposed humans to low levels of NO_2 in our environmental chamber,⁶ and the results of biochemical tests on the blood of human subjects are presented here.

METHODS AND MATERIALS

Healthy young adults volunteered for the studies. Subjects entered the environmental chamber in groups of four or five and underwent a regimen of intermittent light exercise and pulmonary function tests over a two and one-half hour period. Subjects alternated 15 minutes exercise and 15 minutes rest at a level sufficient to double the minute ventilation. All pulmonary function tests were performed during the last 15 minute period. Chamber temperature and relative humidity were maintained at 31°C and 35 percent respectively. These conditions simulate a "typical" smoggy day in Los Angeles. Blood was drawn from each subject immediately after the test period. Each subject was studied in the chamber on three successive days. The environmental chamber atmosphere contained no NO₂ the first day. This constituted a "sham control". The atmosphere contained 1.0 ppm or 2 ppm NO₂ on the second and third days. These two days were designated "exposure days". When all biochemical assays were completed the values obtained for each subject at each concentration during exposure periods were compared to the control values for the same individual. Thus, each subject served as his own control and the statistical method of paired group analysis was utilized. Additional experiments were performed in which subjects followed the three day experimental protocol but breathed only filtered air. These "sham" experiments were conducted to detect any biochemical changes resulting from the chamber procedures *per se*.

Hemoglobin⁷ and Hematocrit⁸ values were determined on each blood sample as a routine hematological procedure. Other observations were chosen because they had been shown to undergo significant changes following ozone (O₃) exposure which may have implications about the subject's response and adaptation to the pollutants. These biochemical assays included measurements of erythrocyte (RBC) membrane fragility⁹ and acetylcholinesterase (AcChase)¹⁰. Erythrocyte levels of reduced glutathione (GSH)¹¹, and the enzymes glucose-6-phosphate dehydrogenase (G6PDH)¹², lactate dehydrogenase (LDH)¹³, glutathione reductase (GSSGase)¹⁴, and glutathione peroxidase (GSHP_x)¹⁵. Lipid peroxidation was determined in whole blood¹⁶ and measurements of serum components include vitamin E,¹⁷ and GSSGase.¹⁸

RESULTS

The paired t test was used to statistically compare the values of each parameter for the first (sham) day, and the two days of NO₂ exposure. Results of experiments in which 1 ppm NO₂ was employed are presented in Table 1. The values are means and standard deviations of results of biochemical measurements on the bloods of 10 subjects. All possible combinations of pairs were tested and statistically significant differences are indicated by the lines and arrows. Results of studies in which 2 ppm NO₂ was employed are shown on Table 2. Table 3 contains data from the three days of sham exposure. All data were treated in a similar way.

DISCUSSION

Previous work has shown that several significant biochemical changes occur in the blood of humans breathing 0.5 ppm O_3 for 2.5 hours.¹⁹ There is much evidence that the primary mechanism for the toxicity of O_3 may involve the formation of free radicals and ozonides, primarily via peroxidation of tissue unsaturated fatty acids.^{16,20} Other changes have been found in lung tissue, including oxidation of sulfhydryl-containing substances in tissues,²¹ and stimulation of pentose shunt and glycolytic enzymes.²² Roehm, et al.²³ found that trace amounts of O_3 and NO_2 caused the rapid oxidation of unsaturated fatty acids *in vitro* and concluded that the oxidation of essential tissue polyunsaturated fatty acids *in vivo* by the air pollutants was likely. These changes are detected as thiobarbituric acid reactive substances in this study. A variety of biochemical changes are brought about in lung by NO_2 inhalation²⁴ and it now appears that alterations also occur in blood.

Erythrocyte membrane AcChase activity was significantly decreased following the first and second day of exposure in both NO_2 atmospheres.^{26,27} The newly formed RBC has a full complement of AcChase and cannot produce any more by enzyme induction since nuclei and ribosomes are lacking. It is normal for the level of AcChase activity to decrease as RBCs age and NO_2 appears to accelerate this process. The erythrocyte membranes did not show an increase in fragility related to sham at either NO_2 concentration in contrast to the response seen with 0.37 ppm or higher levels of O_3 .²⁰ Such antioxidants as vitamin E are thought to

provide protection against oxidant-induced biochemical changes in blood tissue, and the increase detected in serum vitamin E levels following the first NO₂ exposure may have provided some protection. The vitamin E levels were elevated at the end of the first 1 ppm NO₂ exposure but not the second. Evidence exists that mobilization of vitamin E from body depots could possibly result in an increase in membrane-associated vitamin E which would increase the resistance to oxidant induced damage. However, the unsustained increase seen at 1 ppm NO₂ and the failure of a graded response to result from the 2 ppm NO₂ exposure do not suggest that serum vitamin E levels are affected by NO₂ inhalation.

The activity of the enzyme G6PDH showed some increase but the change was not statistically significant. This enzyme participates in the pentose shunt pathway and serves both as an important energy source for lung cells and also provides reduced NADPH required for the reduction of glutathione. Increases in activity would suggest a cellular biochemical response to the injurious effects of NO₂, which includes an increase in the rate of cell metabolism. These data suggest that the respiratory rate of red cells was not significantly stimulated by NO₂ but that some increase in the rate at which cellular reducing substances (NADPH) were produced might have occurred.

The levels of both RBC-GSSGase and RBC-GSH-P_x showed statistically significant increases following 1 ppm NO₂ inhalation but not at the

2 ppm level. These data suggest a stimulatory response to the presence of increased levels of oxidizing free radicals. A similar response is not seen, however, following exposure to the 2 ppm level, possibly because the increased toxicity of the higher NO_2 level inactivates the enzymes. Although somewhat lower than control values, the GSH levels were not significantly different following exposure periods. Glutathione is very sensitive to the presence of oxidants and serves as a cellular antioxidant.²⁸ Glutathione was significantly reduced in RBCs of humans following exposure to 0.5 ppm O_3 while²⁰ GSSGase activity was not changed. The increase in GSSGase and GSHP_x could conceivably have restored any GSH lost through oxidation, and suggests that the mechanisms of RBC cellular damage and response to pollutants may be different for O_3 and NO_2 .

Both NO_2 and O_3 elicit *in vivo* changes in human blood which suggests oxidation of susceptible cell components followed by compensatory responses which may serve to adapt the subject to inhaled oxidants, resulting in enhanced resistance to subsequent exposures.²³ Roehm, *et al.* found that the *in vitro* oxidation of polyunsaturated fatty acids is similar with O_3 and NO_2 but that the process is much more rapid with O_3 . They speculated that the reaction with NO_2 involved free-radical formation at the double bonds which initiate an autocatalytic chain reaction. Ozone on the other hand was thought to attack double bonds directly and produce more stable, though very toxic, ozonides. One of the antioxidant functions of vitamin E is

believed to be to terminate free radical chain reactions in tissues, so it would be expected to provide more protection against NO₂ toxicity than O₃. The results of this experiment suggest this because biochemical changes seen after the first exposure day tend to be greater than after the second. Experiments requiring multiple exposures of humans to O₃ are in progress and preliminary data suggests that biochemical changes are additive and fail to show the attenuated response seen on the second day of NO₂ exposure.

²³ Roehm, *et al.* also found that O₃ produced much more peroxidation than NO₂ when the same concentrations were employed. The *in vivo* toxicity of the two oxidants is also quite different. The biochemical changes detected in this study are comparable to those seen when human subjects were exposed for about the same length of time to 0.37 ppm O₃ or less.³⁰ Biochemical changes in blood are similar at these two concentrations of the respective gases and there is no evidence from these experiments that the irritants produce toxic effects other than by oxidation. The very high oxidation potential of O₃ compared to NO₂ could account for much of the greater toxicity. The lung lesions induced by these two gases occur at the terminal bronchioles of the respiratory tree^{24,31} so their absorption rates are probably similar. Much more evidence is needed before the mechanism of NO₂-induced biochemical changes *in vivo* are understood but the results of this experiment suggest that NO₂ attacks cell membrane lipids, forming free radicals which not only cause damage to the

membrane but also elicit biochemical responses from within the cell. The "no measurable effect", or "threshold" level for NO_2 induced blood biochemical changes has not been verified or determined in humans. The effects of repeated exposure to low NO_2 levels over extended periods of time are also unknown and should be investigated before the possible chronic effects of the air pollutant in human health can be assessed.

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TABLE 1
BIOCHEMICAL CHANGES IN HUMAN BLOOD FOLLOWING 1 PPM NO₂ INHALATION

OBSERVATION	MEAN ± S.D.		
	SHAM	EXPOSURE 1	EXPOSURE 2
RBC Frag. (% hem.)	20.6±3.11	22.9±3.76	17.8±3.93
AcChase (mM/ml/min)	20.7±1.72	20.2±1.58	19.3±1.16
GSH (mg %)	28.4±2.29	27.1±2.70	28.3±2.74
G6PDH (U/gmHb/min)	5.03±0.86	5.18±0.70	5.11±0.64
LDH (U/gmHb/min)	111.0±17.7	106.0±22.6	103.0±17.7
RBC GSSGase (U/ml/min)	2.96±0.267	3.690±0.762	4.020±0.576
RBC GSHP _x (U/ml/min)	8.35±2.44	10.0±3.44	9.52±3.48
Vit. E (mg %)	0.99±0.41	1.08±0.42	0.98±0.45
Lipid Perox. (μgm/ml blood)	0.204±0.070	0.249±0.069	0.249±0.046
Serum GSSGase (mU/ml/min)	24.4±3.54	26.5±3.88	27.8±3.33

RBC Frag. - sensitivity to 2% H₂O₂

AcChase - acetylcholinesterase

GSH - reduced glutathione

G6PDH - glucose-6-phosphate dehydrogenase

LDH - lactate dehydrogenase

RBC GSSGase - erythrocyte glutathione reductase

RBC GSHP_x - erythrocyte glutathione peroxidase

Vit. E - α-tocopherol

Lipid Perox. - thiobarbituric acid reactive substance

— p .05

- - - p .01

n = 10

TABLE 2
BIOCHEMICAL CHANGES IN HUMAN BLOOD
FOLLOWING 2 PPM NO₂ INHALATION

OBSERVATION	MEAN ± S.D.		
	SHAM	EXPOSURE 1	EXPOSURE 2
RBC Frag. (% hem.)	26.45±3.76	23.33±3.32	25.16±1.93
AcChase (mM/ml/min)	21.04±4.48	20.29±4.03	19.35±4.41
GSH (mg %)	29.97±4.36	28.11±3.10	28.50±3.39
G6PDH (U/gmHb/min)	4.30±0.85	4.28±0.98	4.92±0.98
LDH (U/gmHb/min)	105.97±15.91	105.34±13.00	105.05±7.91
RBC GSSGase (U/ml/min)	3.212±0.558	3.172±0.535	3.167±0.611
RBC GSHP _x (U/ml/min)	8.46±1.56	8.93±1.99	8.91±1.71
Vit E (mg %)	1.215±0.271	1.304±0.422	1.350±0.318
Lipid Perox. (ugm/ml)	0.288±0.094	0.344±0.045	0.326±0.072
Serum GSSGase (mU/ml/min)	24.25±4.46	26.06±3.77	25.36±2.11

n = 8

————— p<0.05
----- p<0.01
-x-x- p<0.001

RBC Frag. - erythrocyte fragility to 2% H₂O₂
AcChase - acetylcholinesterase
GSH - reduced glutathione
G6PDH - glucose-6-phosphate dehydrogenase

LDH - lactate dehydrogenase
RBC - GSSGase - erythrocyte
glutathione reductase
RBC-GSHP_x - erythrocyte glutathione
peroxidase
Vit. E - alpha tocopherol
Lipid perox. - thiobarbituric
acid reactive substance

TABLE 3
BLOOD BIOCHEMISTRY FROM HUMAN SHAM EXPOSURE

OBSERVATION	SHAM DAY #	$\bar{X} \pm S.D.$	
RBC Frag. (% hem.)	1	28.54 $\pm$	3.86
	2	24.00 $\pm$	1.83
	3	31.41 $\pm$	2.93
AcChase (mM/ml/min)	1	22.90 $\pm$	4.68
	2	21.98 $\pm$	4.52
	3	21.91 $\pm$	4.34
RBC - GSH (mg %)	1	27.16 $\pm$	2.97
	2	27.87 $\pm$	3.55
	3	27.95 $\pm$	3.16
RBC-Lipid Perox. (μ gm/ml blood)	1	0.289 $\pm$	0.02
	2	0.247 $\pm$	0.009
	3	0.268 $\pm$	0.006
RBC-GSSGase (U/ml/min)	1	3.387 $\pm$	0.319
	2	3.453 $\pm$	0.398
	3	3.405 $\pm$	0.342
RBC-GSHP _x (U/ml/min)	1	8.275 $\pm$	1.172
	2	9.250 $\pm$	1.006
	3	9.837 $\pm$	1.467
G6PDH (U/gm Hb/min)	1	4.219 $\pm$	0.392
	2	4.798 $\pm$	0.806
	3	4.433 $\pm$	0.968
LDH (U/gm Hb/min)	1	105.62 $\pm$	9.28
	2	93.36 $\pm$	15.87
	3	105.58 $\pm$	7.46
Hb (gm %)	1	15.70 $\pm$	0.669
	2	15.66 $\pm$	0.747
	3	15.56 $\pm$	0.715
Ht (%)	1	45.02 $\pm$	3.14
	2	45.65 $\pm$	3.25

n = 8

— = $p < 0.05$

--- = $p < 0.01$

RBC Frag = erythrocyte fragility to 2% H₂O₂

AcChase = acetylcholinesterase

RBC-GSH = erythrocyte reduced glutathione

RBC-lipid perox. = T thiobarbituric acid reactive substance

RBC-GSSGase = erythrocyte glutathione reductase

RBC-GSHP_x = erythrocyte glutathione peroxidase

G6PDH = glucose-6-phosphate dehydrogenase

LDH = lactate dehydrogenase

Hb = hemoglobin

Ht = hematocrit

EXPERIMENTAL STUDIES ON HUMAN HEALTH EFFECTS OF AIR POLLUTANTS
IV. SHORT-TERM PHYSIOLOGICAL AND CLINICAL EFFECTS OF NITROGEN
DIOXIDE EXPOSURE

RUNNING TITLE: PHYSIOLOGICAL EFFECTS OF NITROGEN DIOXIDE

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EXPERIMENTAL STUDIES ON HUMAN HEALTH EFFECTS OF AIR POLLUTANTS

IV. SHORT-TERM PHYSIOLOGICAL AND CLINICAL EFFECTS OF NITROGEN DIOXIDE EXPOSURE

ABSTRACT

Adult male volunteers were exposed to nitrogen dioxide (NO_2) at 1.0 ppm in purified air under conditions simulating ambient photochemical smog exposures (two-hour exposure with intermittent light exercise at 31°C and 35 percent relative humidity). Sham exposures (to purified air alone) served as controls. Exposure effects were assessed by pulmonary physiological tests and by a standardized clinical evaluation. No statistically significant physiological changes attributable to NO_2 exposure were found except for a marginal loss in forced vital capacity after two successive days' exposure (1.5 percent mean decrease, $P < .05$). Reported respiratory and other symptoms were slightly increased with exposure as compared to control, but the change was not significant. Short-term toxicity of NO_2 at peak ambient concentrations appears to be substantially less than that of ozone, but adverse health effects due to NO_2 or its atmospheric reaction products cannot be ruled out at present.

INTRODUCTION

Photochemical smog as experienced in many urban areas is a complex mixture including many oxidizing substances, of which the two typically present in highest concentration are nitrogen dioxide (NO_2) and ozone (O_3). Short-term toxic effects of exposure to O_3 at concentrations attainable in ambient smog are well documented,¹⁻⁷ but comparable information on NO_2 exposure is lacking. In the Los Angeles Basin, an area of high NO_2 pollution, peak ambient concentrations may exceed 1.0 ppm and one-hour average concentrations of 0.8 ppm have been recorded.⁸ Adverse respiratory effects have been reported in humans exposed briefly to concentrations of 5 ppm and less.⁹ This evidence and a variety of findings from animal studies¹⁰ suggest that NO_2 in polluted air may constitute a significant health hazard. This possibility has been investigated in a comprehensive study of short-term health effects in adult male volunteers exposed to NO_2 in purified air under controlled conditions realistically simulating ambient exposures. Results of blood biochemical studies are given in the preceding paper;¹¹ pulmonary physiological and clinical findings are the subject of this report.

METHODS

Exposure and Physiological Testing. The exposure facility, test protocol, and design rationale have been described in detail previously.¹² Subjects (N=16) were exposed in groups of five or six for two hours to 1.0 ppm NO₂ in purified air on two successive days. (During one exposure a failure of the NO₂ generator occurred, requiring use of an alternate generator which produced a mixture of NO₂ and nitric oxide. No differences in effects were found between the group thus exposed and the groups exposed normally.) A control study (exposure to purified air alone) was performed on the day preceding the first exposure to establish baseline values for the various measures. Exposures were performed in a staggered manner for most efficient use of the exposure chamber, with subjects entering and leaving at 45 minute intervals. During exposure each subject exercised for the first 15 minutes in every 30 at approximately 200 kgm/min--sufficient to about double the minute volume of ventilation compared to the resting level. Physiological testing was started after two hours and lasted for approximately 20 minutes, during which exposure continued. The subject then left the exposure chamber and immediately received a clinical examination, during which venous blood was drawn for biochemical analysis. Exposure temperature was $31 \pm 1^{\circ}\text{C}$ and relative humidity 35 percent $\pm$ 4 percent, simulating a smoggy Los Angeles summer day. Subjects were not told the nature of the exposure they were to receive on any given test day, but most were able to distinguish control and exposure conditions on the basis of the characteristic odor of NO₂.

Physiological tests performed included partial and maximum expiratory flow-volume curves, single-breath nitrogen tests, determination of total respiratory resistance and frequency dependence of resistance by forced oscillation applied at the mouth, and determination of thoracic gas volume and airway resistance using a constant-volume body plethysmograph. Flow-volume data collected included forced vital capacity (FVC), one-second forced expiratory volume (FEV_1), peak expiratory flow rate ($\dot{V}_{max}$), and maximum expiratory flow rates at 50 percent and 25 percent FVC from maximum flow-volume curves ($\dot{V}_{50}$, $\dot{V}_{25}$) and partial flow-volume curves ($\dot{V}_{50P}$, $\dot{V}_{25P}$). Closing volume (CV), closing capacity (CC), residual volume (RV), total lung capacity (TLC), and delta nitrogen (ΔN_2) were determined from single-breath nitrogen tests. Total respiratory resistance (R_t) was determined at oscillation frequencies of 3, 6, 9, and 12 Hz. Thoracic gas volume (TGV) and airway resistance (R_{aw}) were determined in the body plethysmograph at functional residual capacity (FRC) and expressed as specific airway conductance (SGaw), equal to $(1/R_{aw})/(TGV)$. Other lung volumes were determined from plethysmographic FRC and inspiratory capacity and expiratory reserve determined by spirometry.

Clinical Assessment. Specific respiratory and other symptoms relatable to exposure were evaluated via a standard interview questionnaire administered by the project physician and were expressed as a semi-quantitative score, as described previously.¹² A total symptom score was determined for each subject on each test day, and scores on control and exposure days were compared to test for clinical effects of exposure.

Subjects. Volunteer subjects were recruited from the project investigators and staff, other hospital workers, and preprofessional students. All were in good general health and had baseline pulmonary function tests within normal limits, although a few had some history of respiratory allergy. Informed consent was obtained from all subjects prior to exposure. Individual characteristics are given in Table 1.

Data Analysis. Individual and group data were compared among control, exposure 1 (initial), and exposure 2 (cumulative) conditions by repeated-measures one-way analysis of variance. As effects found were small and trends were similar in separate groups, a separate analysis was performed in which data for three groups exposed similarly (16 subjects total) were combined to provide greater sensitivity in detecting changes. An additional analysis was performed excluding the data from the subjects who received mixed oxides of nitrogen during exposure 1. No substantial changes in results were produced by excluding these data.

Since symptom-score data were not strictly quantitative, changes in scores between control and exposure were tested by a nonparametric method, the Wilcoxon signed-ranks test.

Pilot Studies. Initial studies, in which the project investigators served as the subjects (N=5), used a different protocol in that single three-hour exposures at 22°C and 45-52 percent relative humidity were employed, only one individual was exposed at a time, and some different pulmonary tests were performed. In particular, esophageal balloons were placed for measurement of transpulmonary pressure and determinations made of static and dynamic lung compliance. Subjects were exposed to 0.5, 1, and in some cases 2 ppm.

RESULTS

Pilot Studies. These results have been previously reported in brief form.¹³ None of the five subjects developed respiratory or other symptoms attributable to exposure or changes in forced-expiratory, single-breath nitrogen, or plethysmographic measures. Mean values for these are given in Table 2A. Subject 7 appeared to develop frequency dependence of dynamic compliance (i.e., reduction of compliance compared to control measurements at frequencies of 60 to 100 breaths/min) during two separate exposures to 1 ppm, but failed to show this effect in one additional exposure at 1 ppm or in another at 2 ppm. None of the other pilot-study subjects showed substantial changes in compliance under any test condition. Measurement of compliance was omitted from subsequent test series because of its instability and technical difficulty, and because other, simpler techniques proved at least equally sensitive in detecting effects of O₃ exposure in related studies.⁶

Pulmonary Physiology. Test results for the three combined subject groups and results of analyses of variance performed on these data are given in Table 2B. As indicated, no changes with exposure were apparent except for a mean loss in FVC of 1.5 percent after the second exposure as compared to control. That this change represents other than a random variation is doubtful due to its small size, its marginal statistical significance, and the relatively large number of statistical comparisons being made. Similarly, data for individuals and for single groups failed to show any marked changes with exposure.

Clinical Assessment. Mean symptom scores for the three combined groups are given in Table 3. While symptoms were increased with exposure, particularly on the second exposure day, the increases were not statistically significant in comparison to control symptom levels. Only five subjects--27, 35, 36, 37, and 39--showed substantially increased symptoms (score increased by three or more points above control) on the second exposure day. Of these five clinically most-sensitive subjects, only one (35) reported substernal pain and coughing reminiscent of typical clinical effects of O_3 .^{6,7} These symptoms were present only to a mild degree. Two others among the five (27 and 36) reported lower-respiratory symptoms (cough or feeling of chest restriction), while all five reported upper-respiratory symptoms (nasal discharge or laryngitis).

DISCUSSION

The results of this study by themselves fail to show convincing evidence of adverse short-term health effects of NO_2 exposure under conditions simulating the worst expected ambient exposure in polluted urban areas. The simultaneous blood biochemical studies¹¹ on the same subjects and others did, however, show statistically significant adverse changes in red cells under these exposure conditions. The biochemical findings taken together with the clinical findings in the apparently most reactive five subjects suggest that NO_2 exposure at 1 ppm may not be considered completely innocuous, even in young healthy adults.

Preliminary evidence suggests that individuals with pulmonary disease are more reactive to O_3 than normals;⁶ if this were true for NO_2 , pulmonary disease patients might be at substantial risk at exposure levels experienced in Los Angeles and other urban areas. Other possible hazards of NO_2 relate to effects of long-term exposure, synergistic effects with other pollutants, and effects in other high-risk groups such as the very young, the very old, and those with preexisting disorders of red-cell metabolism. Little information relevant to these possibilities is available, although one study^{6,7} investigated effects of 0.3 ppm NO_2 in combination with 0.25 or 0.5 ppm O_3 in a small subject sample and found no evidence of enhanced toxicity of the mixture relative to O_3 alone.

Overall results from the series of studies performed in our laboratory allow comparison of the short-term toxicity of NO_2 and O_3 in relatively healthy adult male Los Angeles residents. Marginal physiological effects of O_3 were found at 0.37 ppm and marked effects were found at 0.50 ppm. No effects were detectable at 0.25 ppm. Biochemical effects followed a similar dose-response relationship except that effects at 0.37 ppm were more pronounced relative to 0.25 ppm or to control.⁷ Biochemical effects of 1.0 ppm NO_2 were generally less than those of 0.37 ppm O_3 but greater than those of 0.25 ppm O_3 . As indicated in Table 4, peak NO_2 concentrations in photochemical smog areas of Southern California tend to be similar to, or less than, peak oxidant (primarily O_3) concentrations, thus the apparent markedly higher toxicity of O_3 makes it of greater concern as a health hazard

in acute exposures. Peak levels of NO_2 in low-oxidant areas tend to be higher than in high-oxidant areas, thus NO_2 may be of greater relative health importance in low-oxidant areas. While O_3 and NO_2 coexist in polluted atmospheres, the peak concentrations of each tend to occur at different times of day and different seasons, so their potential for additive or synergistic effects is somewhat less than suggested by the peak concentrations in Table 4.

These studies do not negate the need for adequate controls on emissions of oxides of nitrogen. While the short-term toxic effects of NO_2 at ambient concentrations may be small in healthy subjects, respiratory disease patients are likely to be more sensitive. Furthermore, various products of NO_2 atmospheric reactions may be substantially more toxic than NO_2 *per se*. These include O_3 , peroxyacetyl nitrate (PAN) and related compounds, and inorganic nitrate particulates. A reduction of pollution by these substances is dependent on a reduction of NO_2 pollution.

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TABLE 1 SUBJECT CHARACTERISTICS

SUBJECT NO. (a)	GROUP (a,b)	HEIGHT, CM	WEIGHT, KG	AGE	SMOKER	ALLERGIC HISTORY
1	pilot	172	84	48	former	
3	pilot, 10	180	84	36	former	
4	pilot	188	88	43	current	(c)
7	pilot	175	70	35		(c,d)
12	10	183	75	28		
13	pilot	190	70	28		(c,e)
15	10	165	60	23	current	
17	10	167	65	41		
18	10	178	66	27		
26	13	170	62	28		(e)
27	13	186	77	23		(f)
28	13	178	72	26		
29	13	170	66	24		
30	13	182	77	25		
35	15	170	70	24		
36	15	167	63	23		(c)
37	15	167	66	27	former	
38	15	193	79	27		(c,g)
39	15	178	67	25		
40	15	186	99	23		

NOTES: (a) Studies were interspersed with others in a continuing series, accounting for discrepancies in numbering. (b) During exposure 1 for group 13, the NO₂ generator failed, requiring use of an alternate system produced 1.0 ppm total oxides of nitrogen of which 20-25 percent was NO₂ and 75-80 percent was NO. (c) Hay fever. (d) Rare wheezing episodes. (e) Eczema. (f) Urticaria. (g) Childhood asthma.

TABLE 2A PHYSIOLOGICAL TEST RESULTS IN PILOT
GROUP (N=5) EXPOSED TO 1 PPM NO₂ FOR 3 HR--MEAN±S.D.

<u>MEASURE</u>	<u>CONTROL</u>	<u>EXPOSURE</u>
FVC	5.28±0.63	5.35±0.70
V _{max}	12.2±1.7	12.6±2.0
V ₅₀	5.9±1.1	5.9±0.8
CV (% VC)	15.4±4.7	16.5±5.1
CC (% TLC) (a)	41.5±3.4	41.1±2.6
TLC (b)	7.68±1.38	7.64±1.42
RV (b)	2.41±0.80	2.29±0.76

NOTES: (a) CC = CV from single-breath nitrogen test plus
RV from plethysmographic measurement.
(b) Plethysmographic measurement.

TABLE 2B PHYSIOLOGICAL TEST RESULTS IN 3
COMBINED SUBJECT GROUPS (N=16) EXPOSED TO 1 PPM NO₂
FOR 2 HR--MEAN±S.D. AND ANALYSIS-OF-VARIANCE RESULTS

MEASURE (a)	CONTROL	EXPOSURE 1	EXPOSURE 2	F	df (b)	P
FVC	5.44±0.92	5.40±0.90	5.36±0.90	3.36	2,86	<.05 (c)
V _{max}	12.1±1.68	12.0±1.61	12.0±1.52	<1.0	2,30	NS (d)
V ₅₀	5.51±1.49	5.43±1.57	5.39±1.46	<1.0	2,30	NS
V ₂₅	2.31±0.62	2.28±0.58	2.46±0.82	1.66	2,30	NS
V _{50P}	5.67±1.49	5.97±1.68	5.76±1.23	1.08	2,8	NS
V _{25P}	2.22±0.69	2.44±0.70	2.48±0.74	3.12	2,8	NS
FEV ₁	4.44±0.74	4.41±0.71	4.42±0.70	<1.0	2,76	NS
CV (% VC)	6.0±4.5	5.8±3.8	5.3±4.8	<1.0	2,30	NS
CC (% TLC)	24.4±5.2	24.2±3.6	22.7±4.3	2.30	2,18	NS
ΔN ₂	0.66±0.17	0.67±0.13	0.65±0.15	<1.0	2,92	NS
TLC (e)	6.58±0.96	6.52±0.98	6.56±0.96	2.82	2,90	NS
RV (e)	1.13±0.30	1.15±0.34	1.13±0.28	<1.0	2,90	NS
TLC (f)	7.12±1.02	7.32±1.11	7.30±1.04	1.42	2,30	NS
RV (f)	2.05±1.43	1.85±0.72	1.90±0.77	<1.0	2,30	NS
FRC (f)	3.58±0.61	3.66±0.79	3.69±0.80	<1.0	2,94	NS
R _{aw}	1.20±0.26	1.11±0.35	1.12±0.25	2.56	2,94	NS
SGaw	2.36±0.80	2.37±0.58	2.42±0.64	<1.0	2,36	NS
R _t (3 Hz)	4.26±0.96	4.07±1.20	4.09±1.19	<1.0	2,340	NS
R _t (6 Hz)	3.36±1.10	3.28±0.92	3.62±1.45	1.09	2,328	NS
R _t (9 Hz)	3.09±1.15	2.99±0.91	3.16±1.19	<1.0	2,314	NS
R _t (12 Hz)	2.95±1.11	2.88±0.88	2.90±0.88	<1.0	2,316	NS

TABLE 2B PHYSIOLOGICAL TEST RESULTS IN 3
 COMBINED SUBJECT GROUPS (N=16) EXPOSED TO 1 PPM NO₂
 FOR 2 HR--MEAN±S.D. AND ANALYSIS-OF-VARIANCE RESULTS
 (continued)

- NOTES: (a) Volumes in liters, flows in liters/sec, pressures in cm
 H₂O, ΔN₂ in % N₂ increase per liter expired.
 (b) Degrees of freedom.
 (c) Exposure 2 value significantly different from control value.
 (d) Not significant at .05 level.
 (e) Single-breath method.
 (f) Plethysmographic method.

TABLE 3 SYMPTOM SCORES FOR 3
COMBINED SUBJECT GROUPS (n=16)--CHANGE FROM CONTROL VALUE (a)

	EXPOSURE 1	EXPOSURE 2
Mean change in score	+0.2	+1.4
Scores increased	7	8
Scores unchanged	4	4
Scores decreased	5	4
T (b)	34.0 (NS)	22.5 (NS)

(Mean control score = 1.6)

NOTES: (a) Symptoms scored: cough, sputum production, dyspnea, substernal discomfort, chest restriction, wheezing, laryngitis, nasal discharge, headache, fatigue. Scoring periods: during exposure, remainder of day after exposure completed, following morning. Score for each symptom for each period: 0.5 = minimal, 1 = mild, 2 = moderate, 3 = severe, 4 = incapacitating.

(b) T = smaller sum of ranks for Wilcoxon signed-rank test. T values are not significant (T < 17.5 required for significant increase of score from control, P < .05, 12 pairs of values tested, excluding those with score unchanged.)

TABLE 4 MAXIMUM HOURLY AVERAGE NO₂ AND TOTAL OXIDANT LEVELS
PREVALENT IN REPRESENTATIVE AREAS OF THE LOS ANGELES BASIN, 1971⁸ (a)

LOCATION	POLLUTANT	LEVEL EXCEEDED GIVEN PERCENT OF DAYS		
		1%	10%	50%
West Los Angeles (b)	NO ₂	.46	.27	.12
	Oxidant	.23	.14	.08
Central Los Angeles (b)	NO ₂	.56	.31	.14
	Oxidant	.29	.17	.08
Azusa (b)	NO ₂	.30	.19	.10
	Oxidant	.51	.35	.14
Riverside	NO ₂	.25	.15	.08
	Oxidant	.40	.27	.12

NOTES: (a) Levels (in ppm) exceeded for one hour or more on the indicated percent of days monitored. Ozone is the major contributor to total oxidant concentrations.

(b) Oxidant levels reported for these monitoring stations have been multiplied by 1.3 to account for differences among agencies in calibration techniques.¹⁴ Values tabulated may thus be directly compared with those given elsewhere in this paper.